PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/57, 9/48, C07K 16/40, C12N 5/20, C12Q 1/68, C12N 5/10, C12Q 1/37, A61K 31/70, C07H 19/167, C12N 15/11

A1

(11) International Publication Number: WO 97/38114

(43) International Publication Date: 16 October 1997 (16.10.97)

(21) International Application Number: PCT/EP96/01518

(22) International Filing Date: 9 April 1996 (09.04.96)

(71) Applicant *(for all designated States except US)*: NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basle (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: FONTANA, Adriano [CH/CH]; Lettenstrasse 12, CH-8126 Zumikon (CH). CONSTAM, Daniel, Beat [CH/US]; Harvard University, The Biological Laboratories, 16 Divinity Avenue, Cambridge, MA 02138 (US). TOBLER, Andreas, Rudolf [CH/US]; 857 Partridge Avenue #10, Menlo Park, CA 94025 (US). ALTMANN, Karl-Heinz [DE/CH]; Tannenweg 5, CH-4153 Reinach (CH). SCHLAPBACH, Ralph [CH/CH]; Mattackerstrasse 59, CH-8052 Zürich (CH).

(74) Common Representative: NOVARTIS AG; Patent and Trademark Dept., Klybeckstrasse 141, CH-4002 Basle (CH).

(81) Designated States: AL, AU, BB, BG, BR, CA, CN, CZ, EE, GE, HU, IS, JP, KP, KR, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PUROMYCIN-SENSITIVE AMINOPEPTIDASES

(57) Abstract

The present invention relates to puromycin-sensitive aminopeptidases, antibodies generated against said aminopeptidases, and to means and methods for the production thereof. The invention is also directed to nucleic acids coding for said aminopeptidases, and fragments thereof, to methods of obtaining such nucleic acid molecules or fragments of the invention, and to systems suitable for the expression of such nucleic acids. One particular aspect of this invention relates to deoxyribo- and ribo-oligonucleotides and derivatives thereof, as well as pharmaceutical preparations, therapies, diagnostics and commercial research reagents in relation to disease states which respond to modulation of the synthesis of the enzyme puromycin-sensitive aminopeptidase (PSA).

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

PUROMYCIN-SENSITIVE AMINOPEPTIDASES

The present invention relates to puromycin-sensitive aminopeptidases, antibodies generated against said aminopeptidases, and to means and methods for the production thereof. The invention is also directed to nucleic acids coding for said aminopeptidases, and fragments thereof, to methods of obtaining such nucleic acid molecules or fragments of the invention, and to systems suitable for the expression of such nucleic acids. One particular aspect of this invention relates to deoxyribo- and ribo-oligonucleotides and derivatives thereof, as well as pharmaceutical preparations, therapies, diagnostics and commercial research reagents in relation to disease states which respond to modulation of the synthesis of the enzyme puromycin-sensitive amino peptidase (PSA).

Among the molecular mechanisms which control the cell division cycle, proteolysis has emerged as a key regulatory process enabling cells to pass critical check points. Under basal metabolic conditions, the turnover of intracellular proteins is regulated mainly by non-lysosomal mechanisms involving 26S proteasomes which are multisubunit complexes responsible for the ATP-dependent degradation of poly-ubiquinated and other proteins (Goldberg, A.L., and Rock, K.L. (1992) Nature 357, 375-379; Murakami, Y. et al. (1992) Nature 360, 597-599). Unfortunately, the mechanism is not yet clear as to how proteasomes degrade their substrates into small peptides and amino acids. In particular, those proteasome components which are functionally essential proteases remain to be identified. It is plausible that distal from endoproteolytic steps exopeptidases act to prevent accumulation of peptides and to allow recycling of amino acids.

Aminopeptidases are exopeptidases which catalyze the cleavage of amino acids from the amino terminus of many proteins. Their widespread distribution in plant and animal tissues as well as in bacteria and fungi suggests that they play important roles in various biological processes (for review, see Taylor, A. (1993) Trends Biochem. Sci. 18, 167-172). They generally have a broad substrate specificity, are widely distributed in many tissues and cell types and have been found on cell surfaces, as well as in soluble or secreted forms. Most of the available data on aminopeptidases derive from studies on the metabolism of neuropeptides, such as enkephalins (Tyr-Gly-Gly-Phe-Met (Met-enkephalin) or Tyr-Gly-Gly-Phe-Leu (Leu-enkephalin)). These peptides must be rapidly inactivated at postsynaptic

membranes once they have induced depolarization, and this inactivation is accomplished by enzymatic degradation. Several candidate aminopeptidases have been identified to contribute to neuropeptide degradation (for review, see Brownlees, J., and Williams, C.H. (1993) J. Neurochem. 60, 793-80). A candidate is the ectoenzyme aminopeptidase-N (AP-N), which, although expressed in brain predominantly by microvessels, is detectable also on synaptic membranes (Solhonne, B. et al. (1987) Neurosci. 22, 225-232). A puromycin-sensitive aminopeptidase (PSA), sometimes also referred to as "aminoenkephalinase" (Hui, K.S. et al. (1983) Peptides 4, 639-646) or "aminopeptidase MII" (Hersh, L.B. (1985) J. Neurochem. 44, 1427-1435) has also been considered to play a role (Hersh, L.B. et al. (1980) Nature 286, 160-162; Hersh, L.B., and McKelvy, J.F. (1981) J. Neurochem. 36, 171-178; McLellan, S. et al. (1988) J. Neurochem. 51, 1552-1559). PSA efficiently degrades enkephalins in vitro (Hersh, L.B. et al. (1980) Nature 286, 160-162), and it is present in brain in much higher amounts than AP-N. However, Dyer, S.H. et al. ((1990) J. Neurochem. 54, 547-554) have questioned a function for PSA in neuropeptide metabolism since it was found to be a cytoplasmic protein and thus supposed to be unable to inactivate neuropeptides at synaptic clefts.

The aminopeptidase which is relevant for degrading enkephalins and possibly other neuropeptides has not yet been identified unequivocally, partly because of broad and overlapping substrate specificities and sensitivity to inhibitors. Besides PSA other aminopeptidases may be sensitive to puromycin. PSA is also very sensitive to bestatin induced inhibition, an effect shared with aminopeptidase M and leucine aminopeptidase. So far, a deeper insight into neuropeptide metabolism has been significantly hampered by the non-availability of amino acid and nucleic acid sequence information or a recombinantly produced PSA. This deficit has particularly handicapped the search for therapeutic agents capable of specifically affecting PSA activity and/or expression. For example, the availability of a recombinant PSA in a substantially pure form will contribute to the design of therapeutically useful neuropeptide-based drugs or prodrugs, which are resistant to PSA degradation.

The present invention has achieved the isolation and sequencing of DNA encoding PSA and human and murine PSA in particular, thus providing the amino acid sequence of PSA and enabling the production of substantially pure PSA, e.g. by recombinant DNA techniques. The present invention for the first time enables correlation between PSA

structure and function, thereby providing e.g. means for improved diagnosis, prophylaxis and therapy of PSA-related diseases or disorders.

In one embodiment, the present invention relates to a purified or isolated protein designated puromycin-sensitive aminopeptidase (PSA) which is capable of degrading neuropeptides, particularly enkephalins. It is an additional object of the instant invention to provide immunogens for raising anti-PSA antibodies, as well as to obtain antibodies capable of specifically binding to PSA. Furthermore, the present invention relates to isolated nucleic acids (DNA, RNA) coding for PSA. As used hereinbefore or hereinafter, the term "isolated" is intended to refer to a molecule of the invention in a substantially pure form, obtainable preferably by chemical synthesis and/or by means of genetic engineering. The isolated proteins or nucleic acids of the invention may be useful in ways that the proteins or nucleic acids as they naturally occur are not, such as identification of compounds modulating the expression level or activity of PSA.

In another aspect, the invention provides an isolated nucleic acid that is complementary to, or hybridizes under stringent conditions to, a nucleic acid encoding a PSA of the invention. The invention also relates to antisense oligonucleotides and oligonucleotide derivatives specifically hybridizable with nucleic acids relating to (preferably human) PSA. These oligonucleotides and their derivatives are capable of modulating the synthesis of PSA in cells.

In still another aspect of the invention, the nucleic acid of the invention is DNA and further encompasses a replicable vector comprising the DNA encoding a PSA of the invention operably linked to control sequences recognized by a host transformed by the vector. Furthermore, the invention provides host cells transformed with such vector and a method of using a nucleic acid encoding a PSA of the invention to effect the production of such PSA, comprising expressing such PSA encoding nucleic acid in a culture of the transformed host cells and, if desired, recovering PSA activity from the host cell culture.

The present invention also has diagnostic or therapeutic aspects. For example, it relates to a method in which the presence and/or quantity of a PSA of the invention in a biological sample, e.g. a tissue sample, is determined using a nucleic acid probe based on a nucleic acid sequence described herein, or an anti-PSA antibody. Such method may e.g. be

suitable to predict whether cells are likely to undergo apoptotic cell death when treated with PSA inhibitory compounds or antisense oligonucleotides in vitro or in vivo, or whether biological activities associated with PSA are elevated. Furthermore, overexpression of PSA may be one signal leading to transformation of cells by prolonging cell survival.

In yet another aspect, the present invention relates to assays suitable for the identification of therapeutically effective agents which act by targeting PSA (on protein or nucleic acid level). In particular, the present invention provides a method for modulating PSA activity or function comprising introducing into a cell or organism a PSA inhibitor. Such modulation may influence proteolytic degradation of endogenous PSA substrates, proliferation rate or viability of the cell. The present invention also relates to a method of inducing apoptosis within a cell by inhibiting PSA activity. In such a method activity of PSA is inhibited (either totally or partially) by means of agents or signals which interfere with PSA activity, directly or indirectly. Such agents include e.g. anti-sense sequences or transcriptional modulators which bind PSA-encoding nucleic acid; antibodies or other agents which bind either PSA or a molecule with which PSA must interact or bind in order to carry out its physiological role , e.g. its role in proteolytic degradation, cell proliferation or apoptosis, or agents which degrade or otherwise inactivate PSA. The invention also relates to signals or agents (oligonucleotides, antibodies, peptides) useful in isolation, diagnostic or therapeutic methods described herein.

The protein of the invention is a purified or isolated protein designated puromycin-sensitive aminopeptidase (PSA). According to the invention, isolated PSA means substantially pure, homogenous human or murine PSA which has been identified and is essentially free from other compounds with which it is normally associated in vivo, particularly free from naturally occurring macromolecules. Homogenicity is determined by reference to purity standards known to those skilled in the art. Isolated PSA includes PSA in recombinant cell culture. A preferred isolated protein of the invention is a synthetic or a recombinant protein.

Preferably, substantially pure PSA of the invention is obtained through microbial expression or by chemical synthesis, or a combination of these methods.

In a preferred embodiment, said PSA is the PSA (designated PSA-99) with the amino acid sequence set forth in SEQ ID NO:2, which is obtainable from a human source and

biologically active. PSA of SEQ ID NO:2 is a 875 amino acid protein with the calculated molecular mass of 99 kDa. Equally preferred is the full-length PSA with the amino acid sequence set forth in SEQ ID NO:6, which is obtainable from a murine source and biologically active. This latter protein is considered to be the murine homologue of the before-mentioned human protein. The functional characteristics of the human and murine PSA, including e.g. substrate specificity and inhibitor sensitivity, may be expected to match closely.The above proteins are biologically active in that they exhibit aminopeptidase activity towards suitable substrates. Being puromycin-sensitive aminopeptidases, their amino peptidase activity towards suitable peptide substrates is significantly inhibited by puromycin, as compared to a suitable control in the absence of puromycin. Puromycin (3'[α-amino-p-methoxyhydrocinnamamido]-3'deoxy-N,N-dimethyladenosine; molecular formula: $C_{22}H_{29}O_5N_7$) is an antibiotic which is commercially available (e.g. from Sigma). PSA inhibition by puromycin is considered significant, since 0.16 μM neutralized 81 % of the protease activity, the K_i of puromycin tested on pruified brain PSA being 0.4 μM (Mc. Lellan, St. et al. (1988), J. Neurochem. 51, 1552).

PSA peptidase activity may be detected, and optionally quantified, according to assays well known in the art (see e.g. Brownlees, J. and Williams, C.H. (1993), J. Neurochem 60:793-803; Schnebli, H.P. et al. (1979), Biochim. Biophys. Acta 569:89-98). PSA requires a free N-terminal amino group on the peptidic substrate and sufficient amounts of a divalent cation, particularly Zn^{2+}. For example, detection and quantification of PSA peptidase activity may involve use of amino acid derivatives or peptides containing a detectable leaving group which produces a measurable signal due to PSA-induced hydrolysis of the substrate. For example, the substrate may contain a chromogenic or fluorogenic leaving group, which produces a measurable signal when the peptide bond in which it participates is cleaved by PSA. Suitable PSA substrates include β–naphtylamides and p-nitro anilides of neutral, basic and aromatic, but not acidic, amino acids, e.g. L-Tyr-β–naphtylamide or amino acyl p-nitroanilide, wherein amino is histidine, leucine, methionine, phenylalanine or lysine. A more detailed assay protocol is provided in the Examples.

Also, conventional peptide separation techniques, such as high performance liquid chromatography (HPLC), may be employed to monitor the disappearance of a natural or synthetic peptide or amino acid, or the appearance of cleavage products, on incubation of a

suitable substrate with an enzymatically active PSA of the invention. For example, the cleavage of the Tyr_1-Gly bond in Leu-enkephalin or Met-enkephalin which is radiolabeled in the tyrosine residue may be determined after separating the radiolabelled tyrosine from unreacted enkephalin by reverse phase chromatography.

If desired, PSA enzymatic activity may be assessed in the presence of a PSA enzymatic activity enhancing or inhibiting compound. PSA inhibitors include metal ion chelating agents, such as EDTA, puromycin, bestatin ([2S, 3R-3-Amino-2-hydroxy-4-phenylbutanoyl]-L-leucine; molecular formula: $C_{16}H_{24}N_2O_4$), commercially available e.g. from Sigma), phenantroline, or anti-PSA antibodies.

As used herein, the terms "PSA of the invention" or "protein of the invention" also refer to amino acid mutants of the proteins of SEQ ID NOs: 2 or 6, or glycosylation variants of the nautrally occurring human or murine proteins, splice variants encoded by mRNAs generated by alternative splicing of primary PSA-encoding transcripts, as well as fragments and derivatives of said proteins of SEQ ID NOs: 2 or 6, amino acid mutants or glycosylation variants.

Minor modifications of the primary amino acid sequences of PSA (which may be readily derived from the sequences set forth in SEQ ID NOs:1, 2 , or 5, 6, respectively) may result in amino acid mutants (muteins) or variants which have substantially equivalent properties as compared to the PSA with the amino acid sequences set forth in SEQ ID NOs:2 or 6. Such modifications may be deliberate, as by site-directed mutagenesis, or spontaneous. Variants obtainable by these modifications are included herein, with the provision that they display qualitatively essentially the same functional characteristics as the proteins of SEQ ID NOs:2 or 6. In particular, such mutants should exhibit puromycin-sensitive amino-peptidase activity. For the purposes of this disclosure, such variants are considered as "functional amino acid variants".

Functional amino acid (sequence) variants of the PSAs of SEQ ID NOs:2 or 6 may be substitutional, insertional or deletional. Substitutions, deletions and insertions may be combined to arrive at an amino acid mutant of the invention. Amino acid substitutions are typically of single residues, insertions usually will be on the order of from one to about ten amino acid residues, and deletions will usually range from about one to thirty residues.

Such variants are considered as having essentially the same amino acid sequence as the PSAs of SEQ ID NO:2 or SEQ ID NO:6.

For example, a substitutional amino acid mutant is any polypeptide having an amino acid sequence substantially identical to the sequences set forth in SEQ ID NOs:2 or 6, in which one or more residues have been conservatively substituted with a functionally-similar amino acid residue. Conservative substitutions include e.g. the substitution of one non-polar (hydrophobic) residue, such as methionine, valine, leucine, isoleucine for another, substitution of one polar (hydrophilic) residue for another, such as between glycine and serine, between arginine and lysine, and between glutamine and asparagine. Substitutional or deletional mutagenesis may be employed to eliminate O- or N-linked glycosylation sites. Deletions of cysteine or other labile amino acid residues may also be desirable, for example to increase the oxidative stability of a protein of the invention.

A glycosylation variant of a protein of the invention is an enzymatically active PSA having a glycosylation pattern which is different from that found for native human or murine PSA. For example, a non-glycosylated form of the PSAs of SEQ ID NOs: 2 or 6 may be obtained by deglycosylation of a glycosylated form of such PSA, e.g. by enzymatic removal of the glycosyl residues, or by expression of a nucleic acid encoding a protein of the invention in suitable prokaryotic cells.

As defined herein, fragments of the PSA proteins of SEQ ID NOs:2 or 6 are proteins lacking thirty-one or more consecutive amino acids of said full-length proteins or corresponding amino acid substitutional mutants of the same length. Thus, a fragment of the PSA of SEQ ID NO:2 is a contiguous, uninterrupted sequential fragment of the protein of SEQ ID NO:2 comprising 844 or less, particularly 844 to 8, consecutive amino acids of said PSA, and a fragment of the PSA of SEQ ID NO:8 comprises 889 or less, particularly 889 to 8, consecutive amino acids. Such fragments may be functionally or immunologically equivalent to the corresponding full-length proteins. Functionally equivalent fragments display essentially the same aminopeptidase activity, substrate specificity or inhibitor sensitivity as the above-specified full-length PSA they are derivable from. Immunologically equivalent fragments are fragments comprising at least eight, preferably at least about 20, contiguous amino acids of the amino acid sequences set forth in SEQ ID NOs:2 or 6, and mimicking a

PSA epitope. Such fragments are e.g. suitable for the generation of anti-PSA antibodies, or for investigating an aspect of PSA function isolatedly.

Preferred PSA fragments of the invention include (human) PSA-93, (human) PSA-68, and peptides 1 and 2, as specified in Example 1. In particular, PSA-93 refers to the 825 amino acid-fragment of PSA-99 with the amino acid sequence extending from the amino acid at position 51 (Met) to the amino acid at position 875 (Val) in SEQ ID NO:2, and PSA-68 refers to the 602 amino acid-fragment of PSA-99 with the amino acid sequence extending from the amino acid at position 274 (Met) to the amino acid at position 875 (Val) in SEQ ID NO:2

A derivative of a protein of the invention is a covalent or aggregative conjugate of said protein with another chemical moiety, said derivative displaying essentially the same biological activity as the underivatized protein of the invention.

An exemplary covalent conjugate according to the invention is a conjugate of a protein of the invention with another protein or peptide, such as a fusion protein comprising a protein of the invention, e.g. a PSA of SEQ ID NOs:2 or 6, or a fragment thereof, and a protein tag, such as GST, polyhistidine, or the VSV epitope described in the Examples, or a carrier protein suitable for enhancing the in vivo antigenicity of said protein of the invention. A covalent conjugate of the invention further includes a protein of the invention labelled with a detectable group, e.g. a protein of the invention which is radiolabelled, covalently bound to a rare earth chelate or conjugated to a fluorescent moiety or biotin.

Advantageously, a protein of the invention is obtained by chemical synthesis and/or recombinant DNA techniques. For example, a protein of the invention is obtainable from a nucleic acid which at low stringency hybridizes to the cDNA of SEQ ID NO:5 or to the 0.7 kb NcoI/SpeI fragment of the DNA of SEQ ID NO:1 (bp 1223-1914, cf. Example 2).

Based on the amino acid sequence information provided in SEQ ID NOs:2 and 6 chemical synthesis of a protein of the invention is performed according to conventional methods known in the art. In general, those methods comprise the sequential addition of one or more amino acid residues to a growing (poly)peptide chain. If required, potentially reactive groups, e.g. free amino or carboxy groups, are protected by a suitable, selectively

removable protecting group. Chemical synthesis may be particularly advantageous for fragments of PSA having no more than about 100 to 150 amino acid residues.

The invention also provides a method for preparing a protein of the invention, said method being characterized in that suitable host cells producing the protein of the invention are multiplied in vitro or in vivo. Preferably, the host cells are transformed or transfected with a hybrid vector comprising an expression cassette comprising a promoter and a DNA sequence coding for a protein of the invention which DNA is controlled by said promoter. Subsequently, the protein of the invention may be recovered. Recovery comprises e.g. isolating the protein of the invention from the host cells or isolating the host cells comprising the protein, e.g. from the culture broth.

More specifically, the invention provides a method for producing a protein of the invention, preferably such protein having PSA enzymatic activity, which method comprises growing host cells transformed or transfected with a DNA construct comprising a DNA coding for said protein of the invention, and optionally recovering the PSA enzymatic activity. In particular, the invention provides a method for producing the PSA with the amino acid sequence set forth in SEQ ID NO:2 , or fragment thereof, which method comprises growing prokaryotic or eukaryotic host cells transformed or transfected with a DNA construct comprising a DNA coding for said PSA or fragment, e.g. a DNA with any of the sequences set forth in SEQ ID NOs:1, 3 or 4, and optionally recovering PSA enzymatic activity. The invention also relates to a method for producing the PSA with the amino acid sequence set forth in SEQ ID NO:6, or an enzymatically active fragment thereof, which method comprises growing prokaryotic or eukaryotic host cells transformed or transfected with a DNA construct comprising a DNA coding for said PSA or fragment, e.g. the DNA with the sequence set forth in SEQ ID NO:5, which method comprises growing prokaryotic or eukaryotic host cells transformed or transfected with a DNA construct comprising a DNA coding for said PSA, e.g. the DNA with the sequence set forth in SEQ ID NO:5, and recovering PSA enzymatic activity. If desired, the host cells used for these processes of the invention lack endogenous PSA activity.

Generally, host cells suitable for production of a protein of the invention include eukaryotic cells, e.g. animal cells, particularly mammalian cells, plant cells and fungi, and prokaryotic cells, such as gram-positive and gram-negative bacteria, e.g. E. coli. A protein of the

invention can be produced directly in recombinant cell culture or as a fusion with a signal sequence, preferably a host-homologous signal. Eukaryotic host cells are preferred.

As used herein, in vitro means ex vivo. In vivo includes cell culture and tissue culture conditions, as well as living organisms.

An amino acid mutant, as defined hereinbefore, may be produced e.g. from a DNA encoding a protein of SEQ ID NO:2 or SEQ ID NO:6, which DNA has been subjected to site-specific in vitro mutagenesis resulting e.g. in an addition, exchange and/or deletion of one or more amino acids. While the site for introducing an amino acid variation is predetermined, the mutation per se need not be predetermined, but random mutagenesis may be performed at the target codon or region. For example, substitutional, deletional and insertional variants are prepared by recombinant methods and screened for biological activity, e.g. for PSA enzymatic activity, substrate specificity, inhibitor sensitivity and/or immuno-crossreactivity with the native forms of the protein of the invention, particularly the proteins of SEQ ID NOs:2 or 8. Alternatively, mutants of the invention may be prepared by chemical synthesis using methods routinely employed in the art.

A protein of the invention may be derivatized in vitro or in vivo according to conventional methods known in the art.

A protein of the invention may be used, for example, as immunogen, e.g. to raise PSA specific immunoreagents, in a drug or ligand screening assay, or in a purification method, such as affinity purification of a binding ligand, e.g. an anti-PSA antibody.

A protein of the invention, or a fragment thereof, suitable for in vivo administration and capable of competing with endogenous PSA for an endogenous ligand is envisaged as therapeutic agent, particularly such agent to induce apoptosis, e.g. of tumor cells.

The invention also relates to the use of a protein of the invention for the generation of polyclonal and, preferably, monoclonal antibody, which specifically binds to PSA provided herein. Particularly useful for this purpose is a protein fragment consisting of at least eight or more, preferably eight to about fourty, consecutive amino acids of PSAs of SEQ ID Nos:2 or 6, such as peptides 1 and 2 of Example 1.

In another embodiment, the invention provides polyclonal and, preferably, monoclonal antibodies generated specifically against human or murine PSA. Such antibodies may be useful e.g. for immunoassays including immunohistochemistry, as well as diagnostic and therapeutic applications. For example, antibodies specific for the catalytic site of human or murine PSA are suitable for blocking or interfering with the function of endogenous PSA. Particularly useful are antibodies selectively recognizing and binding to PSA provided herein. The antibodies of the invention can be administered to a subject in need thereof, particularly a human, employing standard methods.

The antibodies of the invention can be prepared according to methods well known in the art through immunization of a mammal using as antigen PSA of the invention (including antigenic fragments thereof and fusion proteins), hereafter referred to as "immunogenic PSA". Immunogenic PSA according to the invention includes e.g. a tagged PSA fusion protein comprising e.g. a polyamino acid tag, or a myc epitope tag, and PSA, or a fragment thereof. A suitable polyamino acid tag is e.g. polyhistidine. Factors to consider in selecting PSA fragments as antigens (either as synthetic peptide or as fusion protein) include antigenicity and uniqueness to the protein. For example, the fragment may be one of the peptides specified in Example 1. Antigenic PSA fragments will usually comprise stretches of hydrophilic amino acid residues. The antibodies as provided by the present invention may be capable of distinguishing between free PSA and PSA associated with the 26S proteasome.

Preferably, a multiple injection immunization protocol is used for immunizing animals with immunogenic PSA of the invention. If desired, immunogenic PSA molecules used to immunize the animal may be fused or coupled to a carrier protein by conjugation using techniques which are well-known in the art. Commmonly used carrier proteins which may be chemically coupled to the molecules include key hole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and a bacterial toxoid, e.g. tetanus or diphteria toxoid. Polyclonal antibodies produced by the immunized animals can be further purified by techniques conventionally used in immunology arts for the purification and/or concentration of polyclonal, or monoclonal antibodies, such as affinity chromatography. For example, antibodies of the invention may be purified by binding to and elution from a matrix to which the peptide used as antigen is bound.

For their specificity and ease of production monoclonal antibodies specific for PSA of the invention are preferred, e.g. for use in detecting PSA in analyte samples (such as tissue and cell line samples). For preparation of monoclonal antibodies, immunization of mouse, rat or goat is preferred. The general method used for the production of hybridomas is well known (Köhler, G. and Milstein, C. (1975), Nature 256, 495). The term antibody as used herein is intended to include intact molecules as well as fragments thereof, such as Fab or $F(ab')_2$ fragments, which are capable of binding the epitopic determinant.

Confirmation of PSA specificity among antibodies of the invention can be accomplished using routine screening techniques known to be suitable for the determination for the elementary reaction pattern of the antibody of interest, such as the enzyme-linked immunosorbent assay (ELISA). For example, it is possible to evaluate the specificity of an antibody of interest without undue experimentation in a competitive binding assay. Such an assay is useful for determining whether the antibody being tested prevents an anti-PSA antibody of the invention from binding to PSA. If the antibody being tested competes with the antibody of the invention, as shown by a decrease in PSA binding by the antibody of the invention, then it is likely that the (monoclonal) antibodies bind to the same or a closely related epitope.

The invention is further intended to include chimeric antibodies of the PSA-specific antibodies described above, or biologically active fragments thereof. As used herein, the term "chimeric antibody" refers to an antibody in which the variable regions of the antibodies derived from one species are combined with the constant regions of antibodies derived from a different species, or alternatively refers to CDR grafted antibodies. Chimeric antibodies are constructed by recombinant DNA technology. In addition, methods of producing chimeric humanized antibody molecules are known in the art and include combining murine variable regions with human constant regions, or by grafting the murine antibody complementary regions (CDRs) onto the human framework. CDRs are defined as the amino acid sequences on the light and heavy chains of an antibody which form the three-dimensional loop structure that contributes to the formation of the antigen binding site. Any of the above described antibodies or biologically active fragments can be used to generate chimeric and CDR grafted antibodies.

The invention also encompasses cell lines (including hybridomas and transfectomas) which produce monoclonal antibodies of the invention. The isolation of cell lines producing monoclonal antibodies of the invention can be accomplished using routine screening techniques which permit determination of the elementary reaction pattern of the monoclonal antibody of interest. Using the monoclonal antibodies of the invention, it is possible to produce anti-idiotypic antibodies which can be used e.g. to screen monoclonal antibodies to identify whether the antibody has the same binding specificity as a monoclonal antibody of the invention. These antibodies can also be used for immunization purposes.

Once produced as described hereinbefore, anti-PSA antibodies may be used diagnostically, e.g. to detect PSA expression in a biological cell or tissue sample or to monitor the level of its expression. PSA may be detected and/or bound using anti-PSA antibodies in either liquid or solid phase immunoassay formats (i.e. when bound to a carrier). Examplary types of immunoassays which can utilized monoclonal antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Specific examples of such immunoassays include the radioimmunoassay (RIA) and the sandwich (immunometric) assay.

The anti-PSA antibodies of the invention may be unlabeled or detectably labelled. There are many different labels and methods of labeling known to those of skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bioluminescent compounds. Another labeling technique which may result in greater sensitivity consists of coupling the antibodies of the invention to low molecular weight haptens, such as biotin. These haptens can then be specifically labeled by means of a second reaction.

The anti-PSA antibodies of the invention may also be useful for in vivo diagnosis, such as to identify a site of overexpression of PSA resulting in prolonged cell survival, e.g. of tumor cells, or to monitor a particular therapy. In using the anti- PSA antibodies of the invention for the in vivo detection of PSA antigen, the detectably labeled monoclonal antibody is given in a dose which is diagnostically effective, meaning that the amount of detectably labelled

anti-PSA antibody is administered in sufficient quantity to enable detection of the site having cells which (over)express PSA.

This invention further covers a nucleic acid (DNA, RNA) comprising an isolated, preferably recombinant, nucleic acid (DNA, RNA) coding for a protein of the invention, or a fragment of such a nucleic acid. In addition to being useful for the production of the above-mentioned recombinant proteins of the invention, such isolated nucleic acids may be useful as probes, thus e.g. readily enabling those skilled in the art to identify and/or isolate nucleic acid encoding PSA. Furthermore, nucleic acid according to the invention is useful e.g. in a method for determining the presence of PSA, said method comprising hybridizing the DNA (or RNA) encoding (or complementary to) PSA to test sample nucleic acid and to determine the presence of PSA. The invention also provides a method for amplifying a nucleic acid test sample comprising a nucleic acid polymerase (chain) reaction with nucleic acid (DNA or RNA) encoding (or complementary to) such PSA.

As defined herein, isolated PSA nucleic acid is intended to embrace nucleic acid encoding a protein of the invention in ordinarily PSA expressing cells where the nucleic acid is in a chromosomal location different from that of natural cells or is otherwise flanked by a different nucleic acid sequence than that found in nature.

Typically, translation of the PSA-99 mRNA is initiated at the second ATG codon (see SEQ ID NO:1, bp 405 to 407) giving rise to a 99 kDa protein consisting of 875 amino acids. Translational start from the first ATG (see SEQ ID NO:1, bp 273 to 275) results in a 919 amino acid protein with a molecular mass of 103 kDa, the amino acid sequence of which is readily deducible from the given DNA sequence.

In particular, the invention provides an isolated DNA molecule encoding a before-mentioned protein of the invention, or a fragment of such DNA. By definition, such a DNA comprises a coding single-stranded DNA, a double-stranded DNA consisting of said coding DNA and complementary DNA thereto, or this complementary (single stranded) DNA itself. Exemplary DNAs encoding proteins of the invention are represented in SEQ ID Nos 1, 3, 4 and 5. SEQ ID NO:3 represents the sequence of a nucleic acid encoding PSA-93, while SEQ ID NO:4 sets forth the sequence of a nucleic acid encoding PSA-68. Especially preferred are DNAs having substantially the same nucleotide sequences as the coding sequences in SEQ ID

NOs. 3 and 4, and particularly SEQ ID NOs. 1 and 5, with the nucleic acids having the same sequences as the coding regions identified in the beforementioned sequence listings being the most preferred. As used herein, nucleotide sequences which are substantially the same share at least about 90 % sequence identity with the specified sequences or coding regions identified therein.

Exemplary nucleic acids of the invention may alternatively be characterized as those nucleic acids which encode an enzymatically active PSA (including isoenzymes or variants of human or murine PSA) and which hybridize to any DNA with the sequence set forth in SEQ ID NOs: 1, 3 ,4 or 5, or a selected portion (fragment) of said DNAs, or nucleic acids which are related to any of the beforementioned specific DNAs via the degenerated genetic code. It is envisaged that a nucleic acid of the invention can be readily modified by nucleotide substitution, nucleotide deletion, nucleotide insertion or inversion of a nucleotide stretch, and any combination thereof. Such modified sequences can be used to produce mutant PSAs which differ from the proteins found in nature. Mutagenesis may be predetermined (site-specific) or random. A mutation which is not a silent mutation must not place sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins.

The PSA nucleic acid sequences provided herein may be employed to identify nucleic acids encoding PSA amino acid variants including allelic or interspecies variants, or PSA isoenzymes with different tissue distribution. A method for identifying such nucleic acid comprises contacting metazoan, particularly mammalian DNA with a nucleic acid probe described herein and identifying DNA(s) which under stringent conditions specifically hybridize(s) to said probe. The phrase" specifically hybdridize" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

Given the guidance provided herein, a nucleic acid of the invention is obtainable according to methods well known in the art. The present invention further relates to a process for the preparation of such nucleic acids. For example, a DNA of the invention is obtainable by chemical synthesis, by recombinant DNA technology or by polymerase chain reaction (PCR), or any combination of these methods. Preparation by recombinant DNA technology may involve screening of a suitable cDNA library or genomic library with a DNA of the

invention. A suitable method for preparing a nucleic acid of the invention may e.g. comprise the synthesis of a number of oligonucleotides, their use for amplification of DNA by PCR methods, and their splicing to give the desired DNA sequence. Suitable libraries are commercially available, e.g. the libraries employed in the Examples, or can be prepared from tissue samples.

As a screening probe or nucleic acid probe mentioned herein, there may be employed a DNA or RNA comprising substantially the entire coding region of a PSA provided herein, or a suitable oligonucleotide probe based on said DNA or RNA. For example, any of the full-length cDNA clones disclosed herein or a DNA fragment thereof, particularly a fragment specified in the Examples, can be used as probe. A suitable oligonucleotide probe capable of specifically hybridizing with a nucleic acid encoding PSA is a single stranded DNA or RNA that has a sequence of nucleotides that includes at least about 14 to about 25 or more contiguous nucleotides that are the same as (or complementary to) any about 14 to about 25 or more contiguous nucleotides of a nucleic acid with any of the sequences set forth in SEQ ID NOs:1, 3, 4 or 5. Preferably, nucleic acid probes of the invention are labeled with suitable label means, e.g. a chemical moiety, for ready detection upon hybridization. For example, a suitable label means is a radiolabel. The preferred method of labelling a DNA fragment is by incorporating ^{32}P-labeled α-dATP with the Klenow fragment of DNA polymerase in a random priming reaction, as is well known in the art. Oligonucleotides are usually end-labeled with ^{32}P-labeled γ-ATP and polynucleotide kinase. However, other methods (e.g. non-radioactive) may also be used to label the fragment or oligonucleotide, including e.g. enzyme labelling and biotinylation.

After screening a nucleic acid sample, such as a suitable library, e.g. with a portion of DNA including substantially an entire PSA-coding region, or a suitable oligonucleotide probe based on such DNA, positive clones are identified by detecting a hybridization signal; the identified clones are characterized by restriction enzyme mapping and/or DNA sequence analysis, and then examined, e.g. by comparison with the sequences set forth herein, to ascertain whether they include DNA encoding a complete PSA (i.e., whether they include translation initiation and termination codons). If partial clones are obtained, they may be used to rescreen the same or a different library to obtain overlapping clones. If the library is genomic, then the overlapping clones may include exons and introns. If the library is a

cDNA library, then the overlapping clones will include an open reading frame. Partial clones may be joined in proper reading frame to produce a full length clone, using routine techniques, such as endonuclease cleavage, ligation and loopout mutagenesis. Complete clones may be identified by comparison with the DNAs and deduced amino acid sequences provided herein.

Furthermore, in order to detect any abnormality of an endogenous PSA, genetic screening may be carried out using nucleotide sequences of the invention as hybridization probes.

Also, based on the PSA nucleic acid sequences provided herein antisense-type therapeutic agents may be designed. The PSA antisense oligonucleotides and derivatives thereof in detail described below may be capable of modulating PSA synthesis in cells. Decreased biosynthesis of PSA leads to a decrease in PSA enzyme activity, finally resulting in cytostasis and potentially apoptotic cell death. The antisense oligonucleotides and derivatives thereof are therefore appropriate for the prevention and therapeutic treatment of diseases that respond to modulation, especially inhibition, of PSA synthesis, and thus of PSA enzyme activity.

More specifically, in accordance with the present invention, oligonucleotides and their derivatives (as well as salts thereof where salt-forming groups are present) are provided that are specifically hybridizable to DNA or RNA, preferably mRNA, deriving from the gene that encodes functional PSA, preferably human PSA. Such an oligonucleotide or oligonucleotide derivative comprises nucleotide units or analogues/derivatives thereof sufficient in number and identity to allow such hybridization. This relationship is commonly denominated as "antisense", and the compounds of the invention are thus antisense oligonucleotides or their derivatives.

Antisense oligonucleotides and their derivatives specifically bind (hybridize) to the complementary sequences on pre-mRNA or mature mRNA, as defined by Watson-Crick base pairing, interfering with the flow of genetic information from DNA to PSA protein.

In one preferred embodiment of the invention, the oligonucleotides or their derivatives are specifically hybridizable to the 3' untranslated region of the mRNA coding for PSA of the invention, especially human PSA, more preferably having a sequence corresponding to that

of human PSA cDNA as described in SEQ ID NO:1. In another preferred embodiment of the invention, the oligonucleotides or oligonucleotide derivatives are specifically hybridizable to the 5' noncoding region of PSA mRNA, more preferably having a sequence corresponding to that of human PSA cDNA as described in SEQ ID NO:1. Most preferred are oligonucleotides and particularly oligonucleotide derivatives corresponding to (preferably of) the sequences described below by SEQ ID NOs: 7-31.

Generally, oligonucleotide derivatives are preferred over oligonucleotides as such.

Within the present specification, the general terms and definitions used herein preferably have the following meanings:

The present oligonucleotides and oligonucleotide derivatives (compounds) can be isomerically pure or they can be present in isomeric mixtures. Thus, if asymmetric phosphorus atoms are present, the compounds can be present as diastereomeric mixtures or as pure diastereomeres.

Some of the oligonucleotides or oligonucleotide derivatives can be present in different tautomeric forms, depending inter alia on the solvent and the ionization status of ionizable groups. Thus, for example, the central group in phosphorothioates [O-(P-SH)(=O)-O] being tautomerizable to [O-(P=S)(-OH)-O] with the more stable form depending, among others, on the solvent and the state of ionization. Within the present specification, the term oligonucleotide derivatives is also to be understood to encompass these tautomeric forms, the presence of which is known to the person skilled in the art.

The prefix "lower" denotes a radical with up to and including 7 carbon atoms, preferably up to and including 4, and most preferably with up to and including 2 carbon atoms.

The term "modulation of the synthesis PSA" preferably means an inhibition of the bio-synthesis of PSA which leads to diminished concentration of the active enzyme in cells.

The term "corresponding" means that the given compound has base pairing characteristics comparable to the nucleic acid sequence referred to, that is, comparable hybridization characteristics.

Hybridization conditions are known in the art and can be found, inter alia, in the reference given below for Northern blotting or in Maniatis et al., "Molecular Cloning - A Laboratory Manual", second edition, Cold Spring Harbor Laboratory Press, 1989, vol. 2, 9.47 to 9.58.

Antisense oligonucleotides or oligonucleotide derivatives according to the invention comprising nucleotide units or analogues/derivatives thereof sufficient in number and identity to allow hybridization preferably have a length that allows specific binding to the target sequence, especially a length corresponding to 5 to 50 nucleotide units, preferably to 10 to 35 nucleotide units, more preferably to 15 to 22 nucleotide units, and most preferably to 18 to 20 nucleotide units.

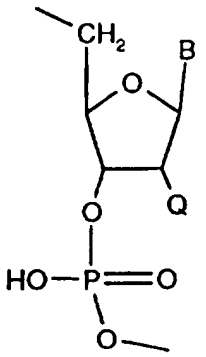
In order to allow also for the inclusion of allelic variants of the human PSA gene and for hybridizable oligonucleotides or oligonucleotide analogues that show minor numbers of mispairing that still allow hybridization, the sequences can vary from those corresponding to the human cDNA (preferably as decribed by Pajunen et al., see above) by some nucleotides or nucleotide analogues; preferably, up to 3 nucleotides or nucleotide analogues can differ in the sequence of a given oligonucleotide or oligonucleotide derivative with respect to the corresponding PSA cDNA, more preferably in the sense of conservative mutations.

A nucleotide unit is a base-sugar or base-sugar analogue combination suitably bound to an adjacent nucleotide unit through phosphodiester or other bonds.

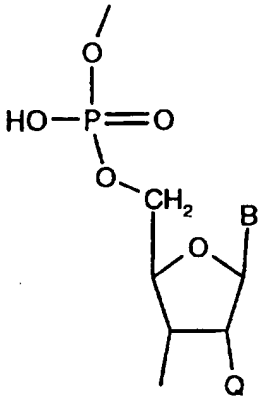
The oligonucleotides or oligonucleotide derivatives according to the invention can be designed to selectively inhibit a given isozyme or a particular set of isozymes, or to inhibit all members of a given family of isozymes of PSA. For example, oligonucleotides may be designed to specifically interact with the proteins herein referred to as PSA-99, PSA-93 and PSA-68.

In the context of this invention, the term "oligonucleotide" refers to an oligonucleotide formed from naturally occurring base radicals and pentofuranosyl (ribosyl or (preferably) 2'-deoxyribosyl) groups or modified forms thereof joined by native phosphodiester bonds, that

is which comprises building blocks of the following formulae I and/or I* wherein Q is H or OH:



(I)



(I*)

wherein B is a radical of a base selected from adenine, cytosine, 5-methylcytosine, thymine and guanine.

The term "oligonucleotide derivative" also refers to synthetic species derived from naturally occurring nucleotide subunits or their close homologs and may also refer to moieties which function similarly to naturally occurring oligonucleotides but which have non-naturally occurring portions, for example at least one building block that differs from the building blocks of a natural oligonucleotide. Thus, oligonucleotides with regard to their backbone may have altered sugar moieties and/or altered inter-sugar linkages, and, with regard to the bases, altered bases may be present.

Such oligonucleotide derivatives are best described as being functionally interchangeable with natural oligonucleotides (or synthesized oligonucleotides along natural lines), but

having one or more differences from natural structure. All such oligonucleotides are encompassed by this invention provided that they function effectively to show the hybridization properties to DNA or RNA deriving from the PSA gene, preferably to mRNA.

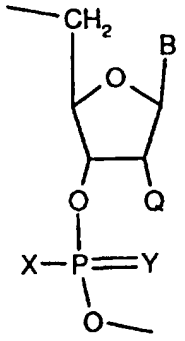
With regard to the backbone, that is to the altered sugar moieties and/or altered inter-sugar linkages, preferred among these are the following types:

- Species derived from naturally occurring nucleotide subunits or their close homologs of formula I or I* wherein B is a base radical as defined below and Q is SH, SCH_3, F, N_3, CN, OCN, OCH_3 or $O(CH_2)_zCH_3$ wherein z is from 1 to about 10; or $O(CH_2CHR_2O)_vR_1$ wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, preferably hydrogen or methyl, R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}-alkenyl, R_2 preferably being hydrogen, methyl, - CH_2-OH, $-CH_2-OCH_3$, and wherein v is from 1 to 4, preferably from 1 to 3.

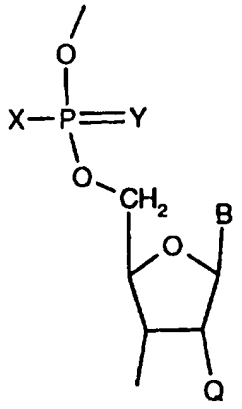
- phosphorothioate and in a broader sense other species such as phosphorodithioate, sulfate, sulfonate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, formacetal, 3'-thio-formacetal, 5'-thioether, hydroxylamine (with $CH_2-NH-O-CH_2$ instead of the phosphodiester bond O-[(HO-)P(=O)]-O-CH_2), methylene(methylimino) (with $CH_2-N(CH_3)-O-CH_3$ instead of the phosphodiester bond); methyleneoxy(methylimino) (with $CH_2-O-N(CH_3)-CH_2$ instead of the phosphodiester bond), methylene-((methylimino)-methylimino) (with $CH_2-N(CH_3)-N(CH_3)-CH_2$ instead of the phosphodiester bond), carbonate, 5'-N-carbamate, amide (with $CH_2-(C=O)-NH-CH_2$ instead of the phosphodiester bond, see International Application WO 92/20823) morpholino-carbamate (see Summerton, J.E. and Weller, D.D., U.S. Patent No: 5,034,506) or peptide nucleic acid (see P.E. Nielsen, M. Egholm, R.H. Berg, O. Buchardt, Science 254, 1497 (1991)) which are known for use in the art (for reviews with references concerning these modified nucleotides, see Milligan et al., J. Med. Chem. 36(14), 1923-37 (1993), and Uhlmann et al., Chemical Reviews 90(4), 543-84 (1990)). In accordance with some preferred embodiments, at least one of the phosphodiester bonds of the oligonucleotide has been substituted with a structure which functions to enhance the ability of the compositions to penetrate into the region of cells where the RNA or DNA whose activity to be modulated is located and in order to avoid extensive degradation of the oligonucleotide derivative due to nucleases that would result in ineffective cleavage products. It is preferred that such substitutions comprise phosphorothioate bonds,

phosphorodithioate bonds, methyl phosphonate bonds, phosphoramidate bonds, amide bonds, boranophosphate bonds, phosphotriester bonds, short chain alkyl or cycloalkyl structures, or heteroatom-substituted short chain alkyl structures, and most especially phosphorothioate bonds.

Preferred of these are oligonucleotide derivatives which (in their nucleotide/nucleotide derivative sequence) comprise at least one of the following units (bivalent radicals) of the formulae given hereinafter, wherein B is a base radical as defined below; Q is SH, SCH_3, F, N_3, CN, OCN, OCH_3 or $O(CH_2)_zCH_3$ wherein z is from 1 to about 10, or $O(CH_2CHR_2O)_vR_1$ wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, preferably hydrogen or methyl, R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}-alkenyl, R_2 preferably being hydrogen, methyl, - CH_2-OH, $-CH_2-OCH_3$, wherein v is from 1 to 4, preferably from 1 to 3, and the other moieties have the meaning given behind the respective formula:



(IIa - IIf)

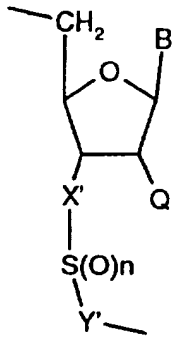


(IIa* - IIf*)

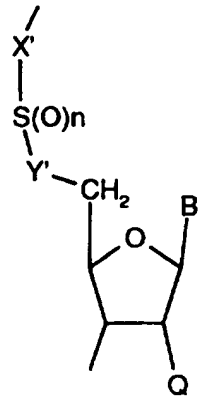
Radical of

formula	type		
(IIa), (IIa*)	phosphorothioate	X = SH	Y = O
(IIb), (IIb*)	phosphorodithioate	X = SH	Y = S
(IIc), (IIc*)	methylphosphonate	X = CH_3	Y = O
(IId), (IId*)	phosphoramidate	X = NH-R	Y = O
(IIe), (IIe*)	boranophosphate	X = BH_3	Y = O
(IIf), (IIf*)	phosphotriester	X = O-R	Y = O

wherein R is lower alkyl;



(IIIa - IIIh)

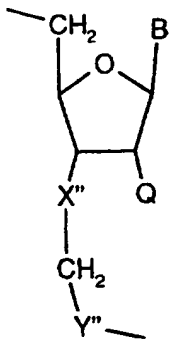


(IIIa* - IIIh*)

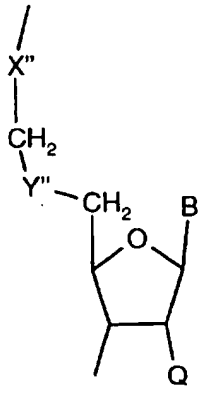
Radical of	type	n	X'	Y'

formula

(IIIa), (IIIa*)	sulfate	2	O	S
(IIIb), (IIIb*	sulfonate	2	O	CH_2
(IIIc), (IIIc*)	sulfamate	2	O	NH
(IIId), (IIId*)	sulfonamide	2	NH	CH_2
(IIIe), (IIIe*)	sulfone	2	CH_2	CH_2
(IIIf), (IIIf*)	sulfite	1	O	O
(IIIg), (IIIg*)	sulfoxide	1	CH_2	CH_2
(IIIh), (IIIh*)	sulfide	0	CH_2	CH_2

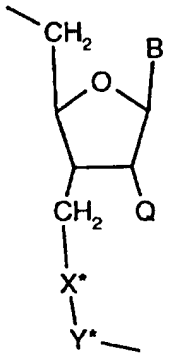


(IVa - IVd)

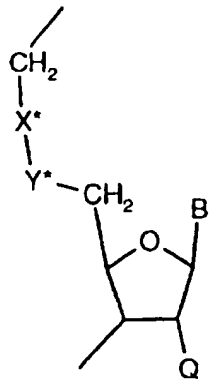


(IVa* - IVd*)

Radical of formula	type	X"	Y"
(IVa), (IVa*)	formacetal	O	O
(IVb), (IVb*)	3'-thioformacetal	S	O
(IVc), (IVc*)	5'-thioformacetal	O	S
(IVd), (IVd*)	thioether	CH_2	S

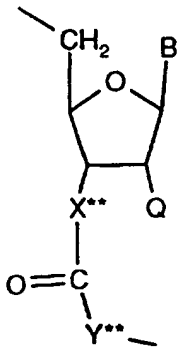


(Va - Vc)

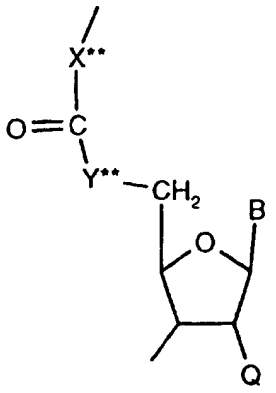


(Va* - Vc*)

Radical of formula	type	X*	Y*
(Va), (Va*)	hydroxylamine	N-H	O
(Vb), (Vb*)	methylene(methyl-imino)	N-CH_3	O
(Vc), (Vc*)	methyleneoxy(methyl-imino)	O	N-CH_3

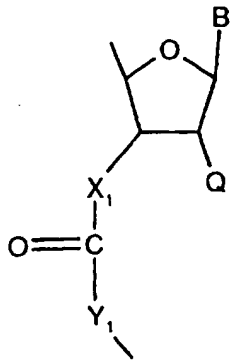


(VIa - VId)

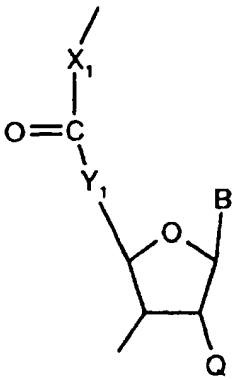


(VIa* - VId*)

Radical of formula	type	X**	Y**
(VIa), (VIa*)	carbonate	O	O
(VIb), (VIb*)	5'-N-carbamate	O	NH
(VIc), (VIc*)	amide	CH_2	NH
(VId), (VId*)	amide II	NH	CH_2

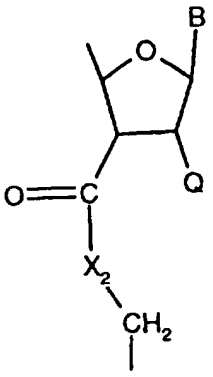


(VII)

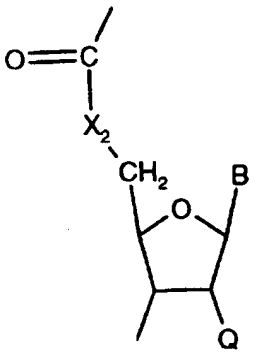


(VII*)

Radical of formula	type	X_1	Y_1
(VII), (VII*)	amide III	NH	CH_2

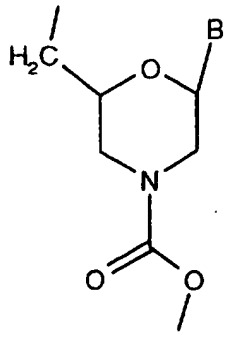


(VIII)

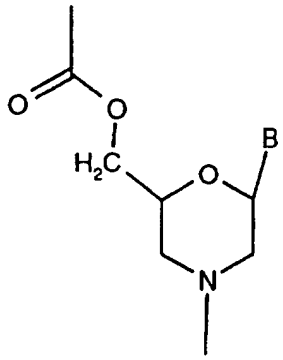


(VIII*)

Radical of formula	type	X_2
(VIII), (VIII*)	amide IV	NH

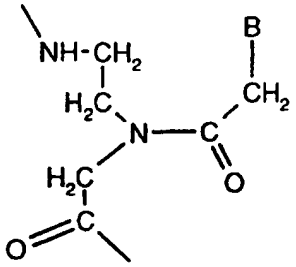


(IX)

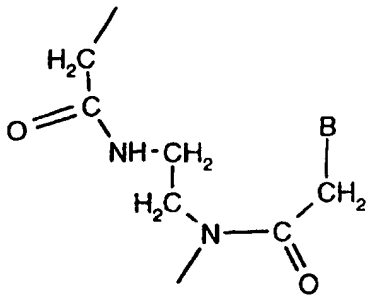


(IX*)

Radical of formula	type
IX, IX*	morpholino-carbamate



(X)



(X*)

Radical of formula	type
X, X*	peptide nucleic acid

The oligonucleotide derivatives can be composed of a combination of these units, or they can preferably comprise only one type of these units with regard to the backbone (sugar moieties and/or inter-sugar linkages) which is present throughout the chain of the respective oligonucleotide derivative, most preferably of the 2'-deoxyribose-phosphorothioate type. At the 5'- and 3'-termini of the respective oligonucleotide derivative molecules, the free valency of the radicals of any of the above formulae I, I*, II to X and II* to X* is bonded preferably to hydrogen if the terminal atom is selected from N, O and S and to hydroxy or an analogue thereof, such as halogen, for example Cl, Br or I, mercapto (SH) or azido (N_3), if the terminal atom is C, more preferably to one of the following residues, but may also (in a broader aspect of the invention) be bound to other conjugated moieties as described below forming conjugates:

In compounds with a terminal moiety of any one of the formulae I, IIa - IIf, IIIa, IIIc, IIIf, IVa - IVd, Va - Vc, VIa - VIc, IX and X*, the 5' terminus is preferably bonded to a terminal OH group, and the 3'-terminus to a hydrogen.

In compounds with a terminal moiety of any one of the formulae I*, IIa* - IIf*, IIIa* - IIIh*, IVa* - IVc*, Va* - Vc*, VIa* - VId*, VII*, VIII* and X, the 5'-terminus is preferably bonded to a terminal hydrogen, and the 3'-terminus to a OH group.

In compounds with a terminal moiety of any one of the formulae IX*, the 5'-terminus is preferably bonded to a terminal OH group which is bonded replacing the terminal -(C=O)-O, and the 3'-terminus to a hydrogen atom.

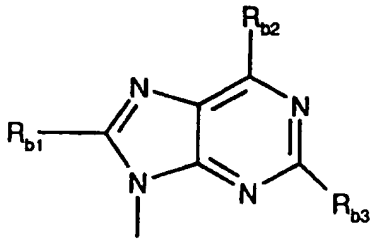
In compounds with a terminal moiety of any one of the formulae IIIb, IIId, IIIe, IIIe*, IIIg, IIIg*, IIIh, IIIh*, IVd*, VId, VII, VIII and VIII*, the 5'-terminus is preferably bonded to a terminal OH group and the 3'-terminus is preferably bonded to an OH group.

In order to allow for modified and improved pharmacokinetic properties, such as enhanced uptake into cells or the oligonucleotides or oligonucleotide derivatives according to the invention can also be conjugated to one or more (then identical or different) additional moieties, for example selected from: A group forming micelles, an antibody, a carbohydrate, a receptor-binding group, a steroid, such as cholesterol, a polypeptide, an intercalating agent, such as an acridine derivative, a long-chain alkohol, a phospholipid and other lipophilic groups.

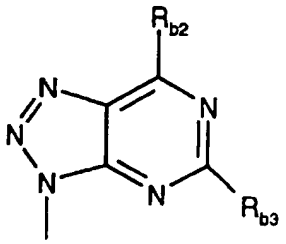
The very most preferred of the oligonucleotide derivatives are those of the phosphoro-thioate type.

B in any of the formulae (Ii) to (Xi) and (Ii*) to (Xi*) ("i" standing for the respective indices in the formulae above, such as, for example, "a", "b" or no index if none is required) is a base radical and is selected from the group comprising a purine radical or an analogue thereof and a pyrimidine radical or an analogue thereof.

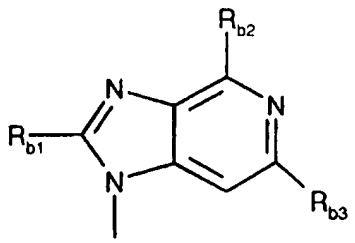
If B is a purine radical or an analogue thereof, it may be a radical of formula XI, XIa, XIb, XIc, XId, XIe or XIf



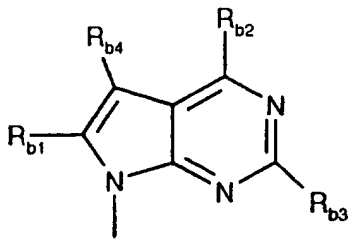
(XI),



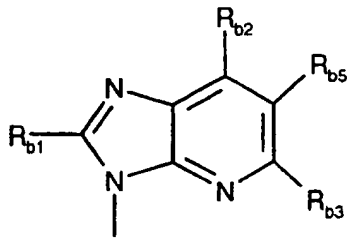
(XIa),



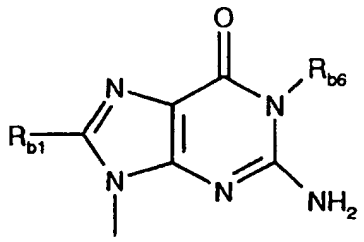
(XIb),



(XIc),

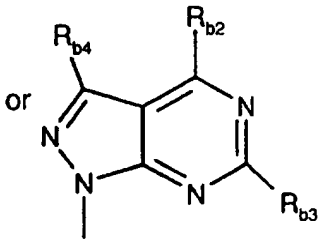


(XId),



(XIe)

or



(XIf),

wherein

R_{b1} is H, Cl, Br, OH or -O-C_1-C_{12}alkyl, and

R_{b2}, R_{b3} and R_{b5} are each independently of the others H, OH, SH, NH_2, $NHNH_2$, NHOH, NHO-C_1-C_{12}alkyl, -N=CH-N(C_1-C_{12}alkyl)$_2$, F, Cl, Br, C_1-C_{12}alkyl, hydroxy-C_1-C_{12}alkyl, amino--C_1-C_{12}alkyl, C_1-C_{12}alkoxy, benzyloxy or C_1-C_{12}alkylthio, the hydroxy and amino groups being present as such or substituted by a protecting group; or phenyl, benzyl, primary amino having from 1 to 20 carbon atoms or secondary amino having from 2 to 30 carbon atoms,

R_{b4} is hydrogen, CN or -C≡C-R_{b7}, and

R_{b6} and R_{b7} are hydrogen or C_1-C_4alkyl.

Protecting groups and processes for derivatising hydroxy groups having such protecting groups are generally known in sugar and nucleotide chemistry and are described in standard text books (see, for example, Greene, B.T., Protective Groups in Organic Synthesis, Wiley Interscience, New York (1991), Sonveaux, E., Bioorganic Chemistry 14:274-325 (1986) or Beaucage, S.L., Iyer, R., Tetrahedron 48:2223-2311 (1992)). If more than one hydroxy group is protected in the respective oligonucleotide or its derivative, the protecting groups may be identical or different.

Protecting groups and processes for derivatising amino groups (as well as imino groups, "amino" groups in the following paragraphs that refer to amino protecting groups also, if possible, meaning imino) having such protecting groups are generally known in sugar, amino acid and nucleotide chemistry and are described, for example, in standard text books (see J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973; Th. W. Greene, "Protective Groups in Organic Synthesis", Wiley, New York 1981, in "The Peptides", Volume 3 (E. Gross and J. Meienhofer, eds.), Academic Press, London and New York 1981; "Methoden der organischen Chemie", Houben-Weyl, 4th edition, Volume 15/I, Georg Thieme Verlag, Stuttgart 1974; and H.-D. Jakubke and H. Jescheit, "Aminosäuren, Peptide, Proteine" ("Amino acids, peptides, proteins"), Verlag Chemie, Weinheim, Deerfield Beach and Basle 1982).

A protected amino group may be protected, for example, in the form of an acylamino, arylmethylamino, etherified mercaptoamino, 2-acyl-lower alk-1-enylamino, silylamino or N-lower alkylpyrrolidinylidene group or in the form of an azido group. Preferred amino-protecting groups are lower alkoxycarbonyl, phenyl-lower alkoxycarbonyl, fluorenyl-lower alkoxycarbonyl, 2-lower alkanoyl-lower alk-1-en-2-yl and lower alkoxycarbonyl-lower alk-1-en-2-yl, with most preference being given to isobutyryl, benzoyl, phenoxyacetyl, 4-tert-butylphenoxyacetyl, N,N-dimethylformamidinyl and/or N-methylpyrrolidin-2-ylidene.

Primary amino (for example in the definition of R_{b2}, R_{b3} and R_{b5}) contains preferably from 1 to 12, and especially from 1 to 6, carbon atoms, and secondary amino (for example in the definition of R_{b2}, R_{b3} and R_{b5}) contains preferably from 2 to 12, and especially from 2 to 6, carbon atoms.

Some examples of alkyl, alkoxy, alkylthio, hydroxyalkyl and aminoalkyl containing preferably from 1 to 6 carbon atoms are methyl, ethyl and the isomers of propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl, and corresponding alkoxy, alkylthio, hydroxyalkyl and aminoalkyl radicals. Alkyl, alkoxy, alkylthio, hydroxyalkyl and aminoalkyl contain especially from 1 to 4 carbon atoms. Preferred alkyl, alkoxy, alkylthio, hydroxyalkyl and aminoalkyl radicals are methyl, ethyl, n- and iso-propyl, n-, iso- and tert-butyl, methoxy, ethoxy, methylthio and ethylthio, aminomethyl, aminoethyl, hydroxymethyl and hydroxyethyl.

The primary amino and the secondary amino may be, for example, radicals of the formula $R_{a1}R_{a2}N$, wherein R_{a1} is hydrogen or, independently, has the definition of R_{a2}, and R_{a2} is C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-alkyl, C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-aminoalkyl, C_1-C_{20}-, preferably C_1-C_{12}- and especially C_1-C_6-hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl, the carbalkoxy group containing from 2 to 8 carbon atoms and the alkyl group from 1 to 6, preferably from 1 to 4, carbon atoms; C_2-C_{20}-, preferably C_2-C_{12}- and especially C_2-C_6-alkenyl; phenyl, mono- or di-(C_1-C_4alkyl or C_1-C_4alkoxy)phenyl, benzyl, mono- or di-(C_1-C_4alkyl or C_1-C_4alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6alkyl, or R_{a1} and R_{a2} together are tetra- or penta-methylene, 3-oxa-1,5-pentylene, -CH_2-NR_{a3}-CH_2CH_2- or -CH_2CH_2-NR_{a3}-CH_2CH_2-, wherein R_{a3} is hydrogen or C_1-C_4alkyl. The amino group in aminoalkyl may be substituted by one or two C_1-C_4alkyl or C_1-C_4hydroxyalkyl groups. The hydroxy group in hydroxyalkyl may be etherified by C_1-C_4alkyl.

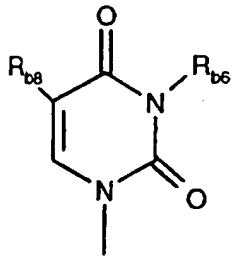
Examples of alkyl are given hereinbefore. Examples of aminoalkyl are aminomethyl, aminoethyl, 1-aminoprop-2-yl or -3-yl, 1-amino-but-2-yl or -3-yl or -4-yl, N-methyl- or N,N-dimethyl- or N-ethyl- or N,N-diethyl- or N-2-hydroxyethyl- or N,N-di-2-hydroxyethyl-aminomethyl or -aminoethyl or -aminopropyl or -aminobutyl. Examples of hydroxyalkyl are hydroxymethyl, 1-hydroxy-eth-2-yl, 1-hydroxy-prop-2- or -3-yl and 1-hydroxy-but-2-yl, -3-yl or -4-yl. Examples of carboxyalkyl are carboxymethyl, carboxyethyl, carboxypropyl and carboxybutyl, and examples of carbalkoxyalkyl are those carboxyalkyl groups esterified by methyl or by ethyl. Examples of alkenyl are allyl, but-1-en-3-yl or -4-yl, pent-3- or -4-en-1-yl or -2-yl, hex-3- or -4- or -5-en-1-yl or -2-yl. Examples of alkyl- and alkoxy-phenyl and alkyl- and alkoxy-benzyl are methylphenyl, dimethylphenyl, ethylphenyl, diethylphenyl, methylbenzyl, dimethylbenzyl, ethylbenzyl, diethylbenzyl, methoxyphenyl, dimethoxyphenyl, ethoxyphenyl, diethoxyphenyl, methoxybenzyl, dimethoxybenzyl, ethoxybenzyl and diethoxybenzyl. Examples of imidazolylalkyl in which the alkyl group preferably contains from 2 to 4 carbon atoms are 1,2-, 1,3- or 1,4-imidazolyl-ethyl or -n-propyl or -n-butyl. R_{a3} is preferably hydrogen, methyl or ethyl.

Preferred examples of primary amino and secondary amino are methyl-, ethyl-, dimethyl-, diethyl-, allyl-, mono- or di-(1-hydroxy-eth-2-yl)-, phenyl- and benzyl-amino, acetylamino, isobutyrylamino and/or benzoylamino.

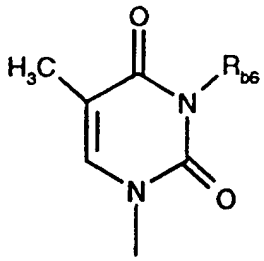
In a preferred form, R_{b1} is hydrogen. In another preferred form, R_{b5} is hydrogen. In a further preferred form, R_{b2} and R_{b3} are each independently of the other H, F, Cl, Br, OH, SH, NH_2, NHOH, $NHNH_2$, methylamino, dimethylamino, benzoylamino, isobutyrylamino, methoxy, ethoxy and methylthio.

Some examples of analogues of the purine series are, in addition to purine, xanthine, hypoxanthine, adenine, N-methyladenine, N-benzoyladenine, 2-methylthioadenine, 2-aminoadenine, 6-hydroxypurine, 2-amino-6-chloropurine, 2-amino-6-methylthiopurine, guanine, N-isobutyrylguanine. Especially preferred are adenine and guanine, and in a broader aspect of the invention 2-aminoadenine, or the base-protected derivatives thereof.

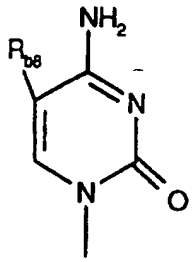
If B in any one of formulae (Ii) to (Xi) and (Ii*) to (Xi*) is a pyrimidine radical or an analogue thereof, it is preferably a uracil, more preferably thymine or cytosine radical or an analogue thereof of formulaXII, XIIa, XIIb or XIIc



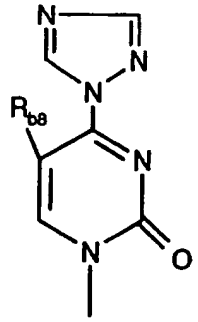
(XII),



(XIIa),



(XIIb) or



(XIIc),

wherein R_{b6} is hydrogen or C_1-C_4alkyl and R_{b8} is H, OH, SH, NH_2, $NHNH_2$, NHOH, NHO-C_1-C_{12}alkyl, -N=CH-N(C_1-C_{12}alkyl)$_2$, F, Cl, Br, C_1-C_{12}alkyl, hydroxy-C_1-C_{12}alkyl, amino-C_1-C_{12}alkyl, C_1-C_{12}alkoxy, benzyloxy or C_1-C_{12}alkylthio, the hydroxy and amino groups being unsubstituted or substituted by a protecting group, or is phenyl, benzyl, primary amino having from 1 to 20 carbon atoms, secondary amino having from 2 to 30 carbon atoms, C_1-

C_{12}alkenyl or C_1-C_{12}alkynyl, and the NH_2 group in formulaXIIb is unsubstituted or substituted by C_1-C_6alkyl, benzoyl or by a protecting group, and the dihydro derivatives of the radicals of formulae XII, XIIa, XIIb and XIIc. R_{b8} in formula XII is preferably hydrogen, C_1-C_6alkyl or C_1-C_6hydroxyalkyl, C_2-C_6alkenyl or C_2-C_6alkynyl, F, Cl, Br, NH_2, benzoylamino or mono- or di-C_1-C_6alkylamino. R_{b8} in formulaeXIIb and XIIc is preferably hydrogen, C_1-C_6alkyl or C_1-C_6alkoxy or C_1-C_6hydroxyalkyl, C_2-C_6alkenyl or C_2-C_6alkynyl, F, Cl, Br, NH_2, benzoylamino or mono- or di-C_1-C_6alkylamino.

R_{b6} is preferably hydrogen or methyl. R_{b8} in formulaXII is preferably H, F, Cl, Br, NH_2, $NHCH_3$, $N(CH_3)_2$, C_1-C_4alkyl, C_2-C_4alkenyl or C_2-C_4alkyn-1-yl. R_{b8} in formulaXIIb and XIIc is preferably hydrogen, C_1-C_4alkyl, especially methyl, C_2-C_4alkenyl, especially vinyl, or C_2-C_4alkyn-1-yl, especially 1-propyn-1-yl, or NH_2, $NHCH_3$ or $(CH_3)_2N$.

Some examples of pyrimidine analogues are uracil, thymine, cytosine, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, dihydrouracil, 5-methylcytosine, 5-propynethymine and 5-propynecytosine, thymine, cytosine and 5-methylcytosine being most preferred.

Salts of oligonucleotides or oligonucleotide derivatives according to the invention are especially acid addition salts, salts with bases or, when several salt-forming groups are present, optionally also mixed salts or internal salts.

Salts are especially the pharmaceutically acceptable, non-toxic salts of oligonucleotides or oligonucleotide derivatives as specified above and below (salts that are non-toxic when applied in the correct dose).

Such salts are formed, for example, from the oligonucleotides or oligonucleotide derivatives having an acidic group, for example a carboxy, phosphodiester or phosphorothioate group, and are, for example, their salts with suitable bases, such as non-toxic metal salts derived from metals of groups Ia, Ib, IIa and IIb of the Periodic Table of Elements, especially suitable alkali metal salts, for example lithium, sodium or potassium salts, or alkaline earth metals salts, for example magnesium or calcium salts, furthermore zinc salts or ammonium salts, also those salts that are formed with organic amines, such as unsubstituted or hydroxy-substituted mono-, di- or tri-alkylamines, especially mono-, di- or tri-lower alkyl-amines, or with quaternary ammonium compounds, for example with N-methyl-N-ethyl-

amine, diethylamine, triethylamine, mono-, bis- or tris-(2-hydroxy-lower alkyl)amines, such as mono-, bis- or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine or tris(hydroxy-methyl)methylamine, N,N-di-lower alkyl-N-(hydroxy-lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)-amine or tri-(2-hydroxyethyl)-amine, or N-methyl-D-glucamine, or quaternary ammonium salts, such as tetrabutylammonium salts. The oligonucleotides and their derivatives having a basic group, for example an amino or imino group, can form acid addition salts, for example with inorganic acids, for example a hydrohalic acid, such as hydrochloric acid, sulfuric acid or phosphoric acid, or with organic carboxylic, sulfonic, sulfo or phospho acids or N-substituted sulfamic acids, such as, for example, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid, gluconic acid, glucaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, nicotinic acid or isonicotinic acid, also with amino acids, such as, for example, the above-mentioned α-amino acids, and also with methanesulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfonic acid, naphthalene-2-sulfonic acid, 2- or 3-phosphoglycerate, glucose-6-phosphate, N-cyclohexylsulfamic acid (with formation of the cyclamates) or with other acidic organic compounds, such as ascorbic acid. Compounds having acidic and basic groups can also form internal salts. If more than one salt-forming group is present, it is also possible that mixed salts are present.

For the purpose of isolation or purification, it is also possible to use pharmaceutically unacceptable salts.

The terms "oligonucleotides", "oligonucleotide derivatives", "compounds" and "salts" also expressly include individual compounds or individual salts.

The oligonucleotides or oligonucleotide derivatives of the invention have valuable pharmacological properties; thus, they are able to decrease the PSA activity in cells by downregulating the synthesis of this enzyme, and can thus, for example, induce apoptosis.

The advantageous pharmacological properties of the oligonucleotides or oligonucleotide derivatives according to the invention can be shown, inter alia, as outlined below:

Using recombinant PSA of the invention, it can be shown that the oligonucleotides or oligonucleotide derivatives of the present invention do not show direct inhibition of PSA. To demonstrate this, PSA activity is recovered from host cells producing an enzymatically active protein of the invention and transformed with an expression plasmid carrying a nucleic acid encoding an enzymatically active protein of the invention, e.g. such plasmid comprising the DNA of SEQ ID NO:1. After incubation of the recovered PSA activity in the absence or presence of oligonucleotides or their derivatives according to the invention, the enzymatic activity is assayed essentially according to known procedures (see e.g. the PSA enzyme activity assays described herein). The antisense oligonucleotides and derivatives thereof according to the invention do not inhibit PSA enzyme activity. Therefore, effects of these oligonucleotides/ their derivatives on cellular PSA activity cannot be attributed to a direct PSA-inhibitory effect of these molecules.

The PSA activity of cell extracts obtained from suitably PSA transfected host cells is determined after incubation of the cells in the absence or presence of oligonucleotides or their derivatives of the present invention according to procedures well-known in the art. Briefly, the following procedure may be used: the PSA activity of cell-free extracts from PSA transfected host cells is determined and the specific PSA activity calculated after protein determination, using e.g. a suitable protein assay kit (as available e.g. from Bio-Rad Laboratories, Richmond, USA; see Bradford, Anal. Biochem. 72, 248-54 (1976). When untransfected cells expressing PSA endogenously, or PSA-transfected host cells, such as COS cells, are exposed to antisense oligonucleotides or their derivatives under suitable conditions, advantageously in the presence of an uptake enhancing agent, such as ®Lipofectin (a 1:1 (w/w) liposome formulation of the cationic lipid N-[1-(2,3-dioleyl-oxy)propyl]-N,N,N-trimethylammonium chloride and dioleyl phosphatidylethanolamine in serum-free medium, the PSA antisense oligonucleotides/their derivatives of the invention are capable of significantly reducing cellular PSA activity. Such test system is suitable for showing that the oligonucleotides and their derivatives according to the invention are specific with regard to the modulation of PSA activity.

Similar test systems may be suitable to demonstrate that the oligonucleotides and especially their derivatives according to the invention inhibit the growth of PSA transfected host cells and induce their apoptosis in tissue cultures.

The compounds of the present invention, due to their ability to diminish the PSA activity in cells by modulation of the amount of PSA being present in the cells due to modulation of its synthesis, especially inhibition thereof, are effective in the treatment of proliferative and especially hyperproliferative diseases, preferably tumor diseases, especially leukemias; tumors of the prostate, such as prostatic carcinoma; tumors of the colon; brain tumors; hyperproliferative skin and epithelial diseases, for example psoriasis, tumors of the epidermis, such as melanoma; (lung cancer, such as lung small-cell carcinoma; or tumors of the urinary tract, especially bladder carcinoma; and any metastases derived therefrom. The antisense oligonucleotides and their derivatives are able, for example, to cause regression of tumors and to prevent the establishment of metastasis and the growth of micrometastases. Both systemic and local application of the antisense oligonucleotides can be envisaged.

Within the following groups of more preferred oligonucleotides and oligonucleotide derivatives of the invention general definitions may be replaced by more specific definitions in accordance with those given above or (especially with regard to definitions of pharmaceutical compositions and methods of use) below.

Preferred is an oligonucleotide derivative that is specifically hybridizable with preferably mRNA, deriving from the gene that encodes PSA, preferably human PSA, comprising analogues of nucleotide units sufficient in number and identity to allow such hybridization, preferably having a length corresponding to 5 to 50 nucleotide units, preferably to 10 to 35 nucleotide units, more preferably to 15 to 22 nucleotide units, and most preferably to 18 to 20 nucleotide units, or a salt of said oligonucleotide derivative where salt-forming groups are present.

More preferred is an oligonucleotide derivative as defined in the last paragraph or an oligonucleotide that is specifically hybridizable to the 3' or with the 5' untranslated regions of the mRNA coding for PSA (especially human PSA), more preferably having a sequence corresponding to that of the DNA of SEQ ID NO:1, and (in a broader aspect of the invention) an allelic variant thereof, preferably with up to 3 nucleotide analogues that differ in the sequence of a given oligonucleotide derivative or oligonucleotide with respect to the

corresponding PSA cDNA, more preferably in the sense of conservative mutations, or a salt thereof if salt-forming groups are present.

Even more preferred is an oligonucleotide derivative or (in a broader aspect of the invention) an allelic variant thereof with up to 3 nucleotide analogues that differ in the sequence of a given oligonucleotide derivative with respect to the corresponding PSA cDNA, more preferably in the sense of conservative mutations, said oligonucleotide derivative or variant having a length corresponding to 5 to 50 nucleotide units, preferably corresponding to 10 to 35 nucleotide units, more preferably corresponding to 15 to 22 nucleotide units, and most preferably corresponding to 18 to 20 nucleotide units, or a salt thereof if salt-forming groups are present, being hybridizable to the 3' untranslated (noncoding) region or the 5' untranslated (noncoding) region of the PSA cDNA (especially human PSA cDNA), an oligonucleotide derivative or (in a broader sense) its allelic variant with up to 3 nucleotide analogues that differ in the sequence of a given oligonucleotide derivative with respect to the corresponding PSA cDNA, having a length corresponding to 5 to 50 nucleotide units, preferably to 10 to 35 nucleotide units, more preferably to 15 to 22 nucleotide units, and most preferably to 18 to 20 nucleotide units, or a salt thereof if salt-forming groups are present.

Most preferred is an oligonucleotide derivative of the sequence described below by any of the SEQ ID NOs from 7 to 31, or a salt thereof of salt-forming groups are present.

In all of the above-mentioned groups of preferred oligonucleotide derivatives, or salts thereof where salt-forming groups are present, those comprising (preferably containing) at least one building block of formula I or I*, wherein Q is SH, SCH_3, F, N_3, CN, OCN, OCH_3 or $O(CH_2)_zCH_3$ wherein z is from 1 to about 10, or $O(CH_2CHR_2O)_vR_1$ wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, preferably hydrogen or methyl, R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}-alkenyl, R_2 preferably being hydrogen, methyl, - CH_2-OH, $-CH_2-OCH_3$, wherein v is from 1 to 4, preferably 1; and B is a base as defined below; of formula IIa to IIf, IIa* to IIf*, IIIa to IIIh, IIIa* to IIIh*, IVa - IVd, IVa* to IVd*, Va to Vc, Va* to Vc*, VIa to VIb, VIa* to VIb*, VII, VII*, VIII, VIII*, IX, IX*, X or of formula X* given above, wherein B is a base radical as defined below, Q is H, OH, SH, SCH_3, F, N_3, CN, OCN, or $O(CH_2)_zCH_3$ where z is from 1 to about 10 or $O(CH_2CHR_2O)_vR_1$ wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, preferably hydrogen or

methyl, R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}-alkenyl, R_2 preferably being hydrogen, methyl, - CH_2-OH, $-CH_2-OCH_3$, wherein v is from 1 to 4, preferably v is 1, most especially hydroxy or preferably hydrogen, and the other moieties have the meanings given behind the respective formula; are even more preferred; and wherein the other building blocks can, in addition to those just mentioned, also comprise building blocks of formula I or I* wherein Q is H or OH and B is a radical of a base selected from adenine, guanine, thymine and cytosine; a nucleotide derivative where no building block of formula I or I*, wherein Q is H or OH and B is a radical of a base selected from adenine, guanine, thymine and cytosine (or a salt thereof where salt-forming groups are present) is present being strongly preferred; and a nucleotide derivative where all building blocks being of the same type with regard to the altered sugar moieties and/or inter-sugar linkages (or a salt thereof where salt-forming groups are present) being the most preferred; a base radical B, if not specified above, being a purine radical or an analogue thereof or a pyrimidine radical or an analogue thereof, preferably a purine radical or an analogue thereof of formula XI, XIa, XIb, XIc, XId, XIe or XIf or a thymine or cytosine radical or an analogue thereof according to formula XII, XIIa, XIIb, XIIc, most preferred being a radical of adenine, guanine, cytosine, 5-methylcytosine or thymine.

In all of the definitions given above, a nucleotide derivative containing only phosphorothioate building blocks of formula IIa and/or IIa*, wherein X is SH and Y is O (the central group [O-(P-SH)(=O)-O] being tautomerizable to [O-(P=S)(-OH)-O] with the more stable form depending, among others, on the solvent and the state of ionization) and wherein B and Q have the given meanings, most preferably B being a radical of adenine, guanine, cytosine or thymine, and Q being OH or preferably H, or a salt thereof, is particularly preferred. Also particularly preferred is an oligonucleotide derivative wherein at least one building block (or more) belongs to the species of formula I or I*, or IIa or IIa*, in which formulas Q is $O(CH_2CHR_2O)_vR_1$, wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, preferably hydrogen or methyl; R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}-alkenyl, R_2 preferably being hydrogen, methyl, - CH_2-OH, - or CH_2-OCH_3; wherein v is from 1 to 4, preferably from 1 to 3; B has the given meanings, most preferably B being a radical of adenine, 2-amino-adenine, guanine, cytosine, 5-methyl-cytosine, 5-propinyl-cytosine, thymine, uracil or 5-propinyl uracil; and all other intersugar linkages, i.e. those which do not involve an above-defined modified sugar moiety, belong to the phosphorothioate type. Most preferred of such derivatives are those, wherein

(as to substituent Q) R_1 is defined as hydrogen or methyl, R_2 is hydrogen, methyl, - CH_2-OH, -CH_2-OCH_3, and v is 1. Furthermore, particularly preferred are phosphorothioate type oligonucleotide derivatives containing at least one, i.e. one or more, amide type radical of formulas VIc, VIc*, VId, VId*, VII, VII*, VIII, or VIII*, preferably an amide- or amide III-type radical (formulas VIc, VIc* and VII, VII*, respectively), wherein B, X**, Y**, X_1 and Y_1 are as defined before, and Q is H, methoxy or methoxyethoxy ($CH_3OCH_2CH_2O$-).

Also particularly preferred are oligonucleotide derivatives wherein an amide building block alternates with a phosphodiester block, and wherein the other variable have the meanings given above.

In any of the groups mentioned above, the oligonucleotide derivative may be free or conjugated, for example to a group forming micelles, to an antibody, a carbohydrate, a receptor-binding group, a steroid, such as cholesterol, a polypeptide, an intercalating agent, such as an acridine derivative, a long-chain alkohol, a phospholipid and/or another lipophilic group, or more of these groups which may also be selected independently from each other.

The oligonucleotides and their derivatives in accordance with this invention may be conveniently and routinely made in analogy to or through methods and using starting materials well-known in the art (for reviews, see, inter alia, Milligan et al., J. Med. Chem. 36(14), 1923-37 (1993) and Uhlmann et al., Chemical Rev. 90(4), 543-84 (1990); see also International Patent Application WO 92/20823, published Nov. 11, 1992), for example by the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including (Applied Biosystems Inc., Foster City, California, USA). Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of the routineer. It is also well known to use similar techniques to prepare other oligonucleotides or derivatives, such as the phosphorothioates, amides and nucleoside modified derivatives (see e.g. International Patent Application WO 95/20597, published August 3, 1995).

The invention also relates to a method of detecting the presence of DNA or RNA which encodes PSA in cells or tissues comprising contacting the cells or tissues with an oligonucleotide derivative as defined above, for example comprising from 5 to 50 nucleotide

units, that is specifically hybridizable with said DNA or RNA, and detecting if hybridization has occurred.

Furthermore, this invention covers a method of diagnosing conditions associated with PSA (over)expression comprising contacting cells or tissues or body fluids from an animal suspected of having a condition associated with PSA (over)expression, or extracts of such samples, with an oligonucleotide or an oligonucleotide derivative as defined above, (preferably) comprising from 8 to 50 nucleotide units, more preferably from 10 to 35 and most preferably from 18 to 20 nucleotide units), specifically hybridizable with selected DNA or RNA deriving from the gene that encodes PSA, most preferably the DNA or mRNA, and determining whether hybridization occurs.

A condition that is associated with PSA (over)expression is, for example, any one of the diseases mentioned above that responds to modulation of PSA expression.

Isolated nucleic acids of the invention can be incorporated into vectors for further manipulation. Such vectors are also provided herein. Specifically, the invention concerns a recombinant DNA which is a hybrid vector comprising at least one of the above mentioned isolated DNAs of the invention, particularly such DNA designated as being preferred.

The hybrid vectors of the invention comprise an origin of replication or an autonomously replicating sequence, one or more dominant marker sequences and, optionally, expression control sequences, signal sequences and additional restriction sites.

Preferably, a hybrid vector of the invention comprises an above described nucleic acid insert operably linked to an expression control sequence, in particular those described hereinafter.

Vectors typically perform two functions in collaboration with compatible host cells. One function is to facilitate the cloning of a nucleic acid that encodes a protein of the invention, i.e. to produce usable quantities of the nucleic acid (cloning vectors). The other function is to provide for replication and expression of the gene constructs in a suitable host, either by maintenance as an extrachromosomal element or by integration into the host chromosome (expression vectors). A cloning vector comprises the DNAs as described above, an origin of

replication or an autonomously replicating sequence, selectable marker sequences, and optionally, signal sequences and additional restriction sites. An expression vector additionally comprises expression control sequences essential for the transcription and translation of the DNA of the invention. Thus an expression vector refers to a recombinant DNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into a suitable host cell, results in expression of the cloned DNA. Suitable expression vectors are well known in the art and include those that are replicable in eukaryotic and/or prokaryotic cells.

Most expression vectors are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in E. coli and then the same vector is transfected into yeast or mammalian cells even though it is not capable of replicating independently of the host cell chromosome. DNA may also be amplified by insertion into the host genome. However, the recovery of genomic DNA encoding PSA is more complex than that of exogenously replicated vector because restriction enzyme digestion is required to excise PSA DNA. DNA can be amplified by PCR and be directly transfected into the host cells without any replication component.

Advantageously, expression and cloning vectors contain a selection gene also referred to as selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that confer resistance to antibiotics and other toxins, e.g. ampicillin, neomycin, methotrexate or tetracycline, complement auxotrophic deficiencies, or supply critical nutrients not available from complex media.

Since the amplification of the vectors is conveniently done in E. coli, an E. coli genetic marker and an E. coli origin of replication are advantageously included. These can be obtained from E. coli plasmids, such as pBR322, Bluescript vector or a pUC plasmid.

Suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up PSA nucleic acid, such as dihydrofolate reductase (DHFR, methotrexate resistance), thymidine kinase, or genes confering resistance to G418 or hygromycin. The mammalian cell transfectants are placed under selection pressure which

only those transfectants are uniquely adapted to survive which have taken up and are expressing the marker.

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to PSA nucleic acid. Such promoter may be inducible or constitutive. The promoters are operably linked to DNA encoding PSA by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native PSA heterologous promoters may be used to direct amplification and/or expression of PSA DNA. However, heterologous promoters are preferred, because they generally allow for greater transcription and higher yields of expressed PSA as compared to native PSA promoter.

Promoters suitable for use with prokaryotic hosts include, for example, the β-lactamase and lactose promoter systems, alkaline phosphatase, a tryptophan (trp) promoter system and hybrid promoters such as the tac promoter. Their nucleotide sequences have been published, thereby enabling the skilled worker operably to ligate them to DNA encoding PSA, using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems will also generally contain a Shine-Delgarno sequence operably linked to the DNA encoding PSA. PSA gene transcription from vectors in mammalian host cells may be controlled by a promoter compatible with the host cell systems, e.g. a promoter derived from the genome of a virus, such as the SV40 virus.

Transcription of a DNA encoding a protein according to the invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector.

The various DNA segments of the vector DNA are operatively linked, i.e. they are contiguous and placed into a functional relationship to each other employing conventional ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required. If desired, analysis to confirm correct sequences in the constructed plasmids is performed in a manner known in the art. Suitable methods for constructing expression vectors, preparing in vitro transcripts, introducing DNA into host cells, and performing analyses for assessing PSA expression and function are known to those skilled in the art. Gene presence, amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern

blotting to quantitate the transcription of mRNA, dot blotting (DNA or RNA analysis), in situ hybridization, using an appropriately labelled probe based on a nucleic acid sequence provided herein, binding assays, immunodetection and functional assays.

The invention further provides host cells capable of producing a PSA protein of the invention and including heterologous (foreign) DNA encoding said protein.

The nucleic acids of the invention can be expressed in a wide variety of host cells, e.g. those mentioned above, that are transformed or transfected with an appropriate expression vector. A protein of the invention may also be expressed as a fusion protein. Recombinant cells can then be cultured under conditions whereby the protein (s) encoded by the DNA of the invention is (are) expressed.

Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-prositive organisms, such as E. coli, e.g. E. coli K-12 strains, DH5α and HB 101, or Bacilli. Further host cells suitable for PSA encoding vectors include eukaryotic microbes, such as filamentous fungi or yeast, e.g. Saccharomyces cerevisiae. Higher eukaryotic cells include insect, amphebian and vertebrate cells, such as HeLa cells or COS cells. The host cells referred to herein include cells in culture as well as cells that are within a host animal. DNA may be stably incorporated into the cells or may be transiently expressed employing conventional methods.

While the DNA provided herein may be expressed in any suitable host cell, e.g. a host cell referred to above, preferred for expression of DNA encoding functional PSA are eukaryotic expression systems, particularly mammalian expression systems, including commercially available systems and other systems known to those of skill in the art.

Stably transfected mammalian cells may be prepared by transfecting cells with an expression vector having a selectable marker gene, and growing the transfected cells under conditions selective for cells expressing the marker gene. To prepare transient transfectants, mammalian cells are transfected with a reporter gene to monitor transfection efficiency. To produce such stably or transiently transfected cells, the cells should be transfected with a sufficient amount of PSA-encoding nucleic acid to form PSA of the

invention. The precise amounts of DNA encoding PSA of the invention may be empirically determined and optimized for a particular cell and assay.

Host cells are transfected or transformed with the above-captioned expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Heterologous DNA may be introduced into host cells by any method known in the art, such as transfection with a vector encoding a heterologous DNA by the calcium phosphate coprecipitation technique, by electroporation or lipofectin-mediated. Successful transfection is generally recognized when any indication of the operation of this vector occurs in the host cell. Transformation is achieved using standard techniques appropriate to the particular host cells used (see, e.g. Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press).

A DNA of the invention may also be expressed in non-human transgenic animals, particularly transgenic warm-blooded animals. Methods for producing transgenic animals, including mice, rats, rabbits, sheep and pigs, are known in the art and are disclosed, for example by Hammer et al. ((1985) Nature 315, 680-683). An expression unit including a DNA of the invention coding for a PSA together with appropriately positioned expression control sequences, is introduced into pronuclei of fertilized eggs. Introduction may be achieved, e.g. by microinjection. Integration of the injected DNA is detected, e.g. by blot analysis of DNA from suitable tissue samples. It is preferred that the introduced DNA be incorporated into the germ line of the animal so that it is passed to the animal's progeny.

Furthermore, a knock-out animal, particularly a mouse, may be developed by introducing a mutation in its PSA gene thereby generating an animal which does not express the functional PSA gene anymore. Such knock-out animal is useful e.g. for studying the role of PSA in metabolism, but in particular for providing a mammalian animal model with a suitable genetic background for introducing and expressing transgenes encoding the homologous human PSA. Expression of human counterpart PSA on a homologous gene knock-out background has e.g. the unique advantage of excluding differences in efficacies of drugs on a given protein (in this case PSA) caused by species-specific sequence differences in said protein.

In a further embodiment, the present invention provides a method for identifying compounds capable of binding to PSA, said method comprising employing a protein of the invention in a binding assay. Such assay may be useful for identification of a synthetic or natural PSA ligand. More specifically, a binding assay according to the invention involves exposure of a protein of the invention, e.g. a PSA of SEQ ID NOs:2 or 6, to a ligand candidate under conditions and for a time sufficient to allow binding of said potential ligand to said protein of the invention, and determining qualitatively and/or quantitatively, whether binding has occurred, e.g. by detecting the complex formed between the ligand and the protein of the invention. Binding of a ligand to the protein of the invention may be analyzed according to suitable conventional methods. Suitability of such method is dependent, for example, on the nature and properties of the particular ligand. Known ligands include puromycin and bestatin.

A preferred binding assay is a competitive binding assay. The principle underlying a competitive binding assay is generally known in the art. Briefly, such a binding assay is performed by allowing a compound to be tested for its capability to compete with a known, suitably labeled ligand, such as L-alanine-2-naphtylamide, for the binding site at a target molecule, i.e. a protein of the invention. A suitably labeled ligand is e.g. a radioactively labeled ligand or a ligand which can be detected by its optical properties, such as absorbance or fluorescence. After removing unbound ligand and test compound the amount of labeled ligand bound to the protein of the invention is measured. If the amount of bound labeled ligand is reduced in the presence of the test compound, said compound is very likely to bind to the target molecule, i.e. the protein of the invention. Compounds binding to the target protein of the invention may modulate a functional property of PSA and may thereby be identified as an PSA activator or inhibitor in a functional assay. A competitive binding assay may be performed e.g. with extracts or lysates of suitably transformed or transfected host cells expressing the protein of the invention, or with a soluble or immobilized form of such protein of the invention.

In yet another aspect, the present invention relates to a functional assay, which is suitable for detection of a change of a physical-chemical property of PSA, such as conformation, binding affinity for associatable molecules, and particularly enzymatic activity. Such functional response may result e.g. from the interaction of the compound to be tested with

PSA of the invention, and may affect e.g. the biological activity of other proteins influenced by PSA within a cell expressing functional PSA (as compared to a negative control). Based on the information provided herein, those of skill in the art can readily identify an assay suitable for detecting such change in the activity of another protein indicative of the expression of PSA.

More specifically, the present invention also provides a method suitable for identifying a compound or agent which modulates the biological activity of PSA, said method comprising contacting a protein of the invention, particularly a PSA of SEQ ID NOs: 2 or 8, with at least one compound or agent, whose ability to modulate the activity of PSA is sought to be investigated, and determining the change of PSA activity of said protein caused by the component or agent.

A component or agent which modulates the activity of PSA includes a compound or signal that is capable of altering the response pathway mediated by functionally active PSA within a cell (as compared to the absence of PSA). Modulation of PSA activity particularly refers to modulation of PSA catalytic activity (aminopeptidase activity), ligand (substrate) binding affinity and/or kinetics, PSA regulatory function for cell survival and gene transcription. Assays for determining a change in any of these properties are well-known in the art. Such assays enable identification of compounds or agents which stimulate or inhibit PSA activity and/or level.

Host cells expressing a nucleic acid of the invention are e.g. useful for drug screening, and the present invention encompasses a method for identifying a compound or signal which modulates the biological activity of PSA. More specifically, the invention covers an assay for identifying compounds which modulate the activity of PSA, said assay comprising:
- contacting cells producing functionally active PSA and containing heterologous DNA encoding PSA with at least one compound to be tested for its ability to modulate the activity of PSA, and
- monitoring said cells for a resulting change in PSA activity.
Preferred such cells are suitably manipulated mammalian cells. Changes suitable to be monitored and induced by a modulation of PSA biological activity include cell cycle arrest during mitosis and apoptotic death.

In particular, the present invention relates to a method suitable for identifying a compound or agent which decreases or inhibits the biological activity of PSA (PSA inhibitor). Such a method comprises contacting a protein of the invention, particularly a PSA of SEQ ID NOs: 2 or 6, with at least one compound or agent, whose ability to decrease or inhibit PSA activity is sought to be investigated, and determining the decrease or inhibition of PSA acitivity of said protein caused by said component or agent. Advantageously, inhibitors are identified by their ability to reduce PSA peptidase activity (as compared to a suitable control).

For example, a change in PSA aminopeptidase activity may be determined according to assays well-known in the art. PSA induced proteolytic degradation of a suitable PSA substrate may be analyzed qualitatively or quantitatively, e.g. using an assay mentioned herein. To this end, PSA may be produced using an appropriate expression system, such as yeast cells, E. coli cells, insect cells or, preferably, mammalian cells transformed with suitable expression vectors containing PSA-encoding heterologous DNA of the invention. PSA enzymatic activity is recovered, e.g. in a cell extract or cell lysate, and exposed to a test compound under conditions allowing interaction of said compound with PSA of the invention. PSA peptidase activity towards a suitable PSA substrate is determined, e.g. a substrate referred to herein. Additionally, such assay may employ a PSA inhibitor. For example, puromycin, bestatin or a metal chelator, e.g. EDTA or phenanthroline, may be used as inhobitors of PSA function, pepstatin or phopshoramidon as negative controls.

If a test compound has been found to inhibit PSA enzymatic acitvity, dose-dependency of the inhibition should be determined. In response to increasing amounts of a compound inhibiting the PSA activity, corresponding decreases in proteolytic degradation of the PSA substrate should be detectable.

An above-described cellular assay is particularly useful to identify PSA-inhibitors capable of inhibiting cell growth and inducing apoptosis. Methods for investigating these phenomenons are well-known in the art and include those described in detail in the Examples. More specifically, due to inhibition of PSA activity, a PSA-inhibitor may be capable of inhibiting proliferation and inducing apoptosis in cells expressing PSA. In cells requiring PSA during mitosis, a PSA-inhibitor may be capable of arresting the cell cycle during mitosisis (G2 and/or M phase). Such an arrest of the cell cycle during G2/M phase may be assessed

employing methods well-known in the art, e.g. conventional methods involving quantification of cellular DNA synthesis or protein synthesis, particularly a method described in detail in Example 2. A PSA-inhibitor may also be able to induce cells to die via apoptosis. Such cells show the morphologic characteristics of apoptosis, such as chromatin condensation around the margin of the nuclei, membrane blebbing and endonucleolytic cleavage of genomic DNA (DNA fragmentation). These characteristics of apoptotic cells may be investigated using conventional methods readily apparent to those skilled in the art, e.g. the methods described in more detail in the Examples.

Generally, assay methods require comparison to various controls. A change in PSA activity is said to be induced by a test compound if such an effect does not occur in the absence of the test compound. An effect of a test compound on a functionally active protein of the invention is said to be mediated by said protein, if this effect is not, or only to a little extent, observed in the absence of said protein, e.g. in cells which do not produce a protein of the invention.

Conditions and times sufficient for interaction of a compound or agent with PSA may vary, however, conditions generally suitable for interaction occur between about 4°C and about 40°C, preferably at about 37°C, in a suitable buffer solution or within a pH range of between 5 and 9, preferably between 6.5 and 8. Sufficient time for the interaction will generally be between about 15 min and about 24 h after exposure. Suitable conditions are e.g. those described in the Examples.

In an assay of the invention, the protein of the invention may be used in a soluble, immobilized or cellular form. If used in an immobilized form, the protein of the invention is attached to a solid support. To obtain a cellular form of the protein of the invention, it is produced by a suitably transformed host cell which is employed in the assay. Preferably, the protein used in such assay is a recombinantly produced protein. The assays provided herein enable identification and design of PSA-specific compounds, particularly molecules specifically binding to PSA (PSA-ligands).

The assays of the invention may be useful to identify compounds or signals which are capable of acting as therapeutic agents in a mammal in need thereof, and which are effective against a PSA-dependent disease or disorder. Thus, the information provided

herein renders possible e.g. identification of drugs capable of inhibiting aberrant cell proliferation (antineoplastic drugs) or drugs capable of inducing apoptosis. Such drugs may be suitable as therapeutic agents against hyperproliferative disorders, such as benign and malignant tumors, and psioriasis. Apoptosis occurs, for example, in malignant tumors, whereby growth of tumors is often delayed. Increased apoptotic effects are found in tumors, which respond to irradiation, cytotoxic chemotherapy and hormonal ablation. Furthermore, apoptosis-inducing PSA-inhibitors may be useful therapeutic agents to induce endometric and prostatic regression (endometriosis, prostata-hypertorphy), to reduce the size of adipose cells (obesity), and in immunosuppressive disorders.

In a further aspect, the invention provides a method of reducing the viability of a proliferating mammalian cell exhibiting PSA activity and to a method of arresting growth of such cells. Uncontrolled cell growth of proliferating mammalian cells can be regulated by decreasing the (expression) level or activity of PSA in the proliferating cell or cell population, thereby inducing cell cycle arrest and apoptosis. Proliferating mammalian cells susceptible to apoptosis mediated by PSA inactivation or PSA depletion include, for example, cancer cells and other neoplasia. Essentially, decrease of PSA level or activity will only lead to cell cycle arrest or apoptosis in proliferating cells, but not in non-proliferating or arrested cells, such as normal terminally differentiated cells.

To modulate the level of PSA, cellular PSA synthesis may be inhibited through the use of antisense or triplex oligonucleotides, analogues or expression constructs. This method entails introducing into the cell a nucleic acid sufficiently complementary in sequence so as to specifically hybridize to the PSA-encoding gene or message. More specifically, this invention also relates to a method of modulating the expression of PSA comprising contacting tissues or cells containing the gene with an oligonucleotide derivative comprising from 5 to 50 nucleotide units, preferably from 10 to 35, most preferably from 18 to 20 nucleotide units, specifically hybridizable with selected DNA or RNA deriving from the PSA gene, most preferably the corresponding DNA or mRNA.

When cells or tissues are to be contacted with an oligonucleotide or oligonucleotide derivative according to the invention in vitro, conditions where an uptake enhancing agent is present are preferred or even necessary. Uptake enhancing agents are, for example, liposome formulations, such as ®Lipofectin (a 1:1 (w/w) liposome formulation of the cationic

lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleyl phosphatidylethanolamine (adding up to 1 mg/ml) in membrane filtered water; GIBCO BRL Life Technologies Inc., Gaithersburg, USA); ®Lipofectamine (a 3:1 (w/w) liposome formulation of the polycationic lipid 2,3-dioleyloxy-N-[2-(sperminecarboxyamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA) and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) in membrane filtered water (GIBCO BRL, USA); N-[1-(2,3-Dioleoyloxy)-propyl]-N,N,N-trimethylammoniumsulfate (Boehringer Mannheim GmbH, FRG), which are present preferably in concentrations ranging from about 0.2 to about 20 µg/ml, for example about 5 µg/ml, in the respective in vitro experiments.

The present invention relates also to pharmaceutical compositions comprising an oligonucleotide or an oligonucleotide derivative with the properties according to the invention as active ingredient. Especially preferred are compositions for enteral, especially oral, or parenteral administration. The compositions comprise the active ingredient on its own or, preferably, together with a pharmaceutically acceptable carrier. The dose of the active ingredient depends on the disease to be treated, and on the species, age, weight and individual condition, as well as the method of administration.

Preferred is a pharmaceutical composition that is suitable for administration to a warm-blooded animal, especially man, suffering from a disease that responds to the modulation of PSA synthesis; for example a proliferative and especially hyperproliferative disease, preferably a tumor disease, especially a leukemia; a tumor of the prostate, such as prostatic carcinoma; a tumor of the colon; a brain tumor; a hyperproliferative skin or epithelial disease, for example psoriasis; a tumor of the epidermis, such as melanoma; (preferably) a lung cancer, such as lung small-cell carcinoma; and/or (most preferably) a tumor of the urinary tract, especially bladder carcinoma; and any metastases derived therefrom; comprising an amount of the active ingredient, or of a salt thereof if salt-forming groups are present, that is effective in the modulation of the synthesis of PSA, preferably in the treatment or prophylaxis of the mentioned diseases, together with at least one pharmaceutically acceptable carrier.

The pharmaceutical compositions comprise from approximately 0.0001 % to approximately 95% active ingredient, dosage forms that are in single dose form preferably comprising from

approximately 0.001 % to approximately 20 % active ingredient, and dosage forms that are not in single dose form preferably comprising from approximately 0.001 % to approximately 10 % active ingredient. Unit dose forms, such as dragées, tablets, ampoules or capsules, comprise from approximately 0.0005mg to approximately 0.5 g of the active ingredient, preferably from 0.005 mg to approximately 20 mg.

The pharmaceutical compositions of the present invention are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, confectioning, dissolving or lyophilising processes. For example pharmaceutical compositions for oral administration can be obtained by combining the active ingredient with one or more solid carriers, where necessary granulating a resulting mixture and processing the mixture or the granules, if desired or appropriate with the addition of further excipients, to form tablets or dragée cores.

Suitable carriers are especially fillers, such as sugars, e.g. lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, e.g. tricalcium phosphate or calcium hydrogen phosphate, and binders, such as starches, e.g. corn, wheat, rice or potato starch, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethyl-cellulose and/or polyvinylpyrrolidone, and/or, if desired, disintegrators, such as the above--mentioned starches, and also carboxymethyl starch, crosslinked polyvinylpyrrolidone or alginic acid or a salt thereof, such as sodium alginate. Additional excipients are especially flow conditioners and lubricants, e.g. silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol, or derivatives thereof.

Dragée cores may be provided with suitable, optionally enteric, coatings, there being used, *inter alia*, concentrated sugar solutions which may comprise gum arabic, talc, polyvinyl-pyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents or solvent mixtures, or, for the preparation of enteric coatings, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethyl-cellulose phthalate. Dyes or pigments may be added to the tablets or dragée coatings, e.g. for identification purposes or to indicate different doses of active ingredient.

Orally administrable pharmaceutical compositions are also dry-filled capsules consisting of gelatin, and also soft sealed capsules consisting of gelatin and a plasticiser, such as

glycerol or sorbitol. The dry-filled capsules may contain the active ingredient in the form of granules, for example in admixture with fillers, such as corn starch, binders and/or glidants, such as talcum or magnesium stearate, and, where appropriate, stabilisers. In soft capsules, the active ingredient is preferably dissolved or suspended in suitable liquid excipients, e.g. fatty oils, ®Lauroglycol (Gattefossé S.A., Saint Priest, France), ®Gelucire (Gattefossé S.A., Saint Priest, France) or sesame oil, paraffin oil or liquid polyethylene glycols, such as PEG 300 or 400 (Fluka, Switzerland), or polypropylene glykols, to each of which stabilisers or detergents may also be added.

Other oral forms of administration are, for example, syrups prepared in customary manner that comprise the active ingredient e.g. in suspended form and in a concentration of approximately from 0.001 % to 20 %, preferably approximately 0.001% to about 2%, or in a similar concentration that provides a suitable single dose when administered, for example, in measures of 5 or 10 ml. Also suitable, for example, are powdered or liquid concentrates for preparing shakes, e.g. in milk. Such concentrates can also be packed in single-dose quantities.

Transdermal Delivery Systems are possible, especially with neutral active ingredients according to the invention. Suitable formulations comprise, for example, about 0.0001% to about 2% by weight of active ingredient. In a preferred aspect, there are provided formulations which comprise about 2 % to 99.9999 % (or the balance to 100 %) of a short chain aliphatic alcohol. Suitable alcohols include ethanol, isopropanol, propylene glycol and glycerol. In a more preferred aspect, these formulations may additionally comprise a flux enhancer. Suitable flux enhancers include, for example, decylmethylsulfoxide, dimethylsufoxide as well as cyclic ketones, lactones, anhydrides and esters. Some of these flux enhancers also increase retention of the active ingredient and thus act to increase the concentration of it in the skin itself. For formulations for direct (local) treatment, such as topical application to the skin, it is preferred to use a flux enhancer which not only maximizes transdermal flux, but increases retention of the active ingredient in the skin. Certain cyclic ketone and lactone enhancers have been reported to increase local retention as well and, thus, comprise a preferred class of enhancers for topical administration of the active ingredient. In formulations for systemic treatment, it is preferable to use a flux enhancer which maximizes flux with a minimal local retention of the active ingredient.

Suitable rectally administrable pharmaceutical compositions are e.g. suppositories that consist of a combination of the active ingredient with a suppository base. Suitable suppository bases are e.g. natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols or higher alkanols.

For parenteral administration (which is preferred) there are suitable, especially, aqueous solutions of an active ingredient in water-soluble form, e.g. in the form of a water-soluble salt, or aqueous injection suspensions that comprise viscosity-increasing substances, e.g. sodium carboxymethylcellulose, sorbitol and/or dextran, and, where appropriate, stabilisers. The active ingredient, where appropriate together with excipients, may also be in the form of a lyophilisate and may be made into a solution prior to parenteral administration by the addition of suitable solvents.

Solutions as used e.g. for parenteral administration may also be used as infusion solutions.

The invention relates also to a method of treating the above-mentioned pathological conditions. For this purpose, an active ingredient of the present invention, or a pharmaceutically acceptable salt thereof, may be administered prophylactically or therapeutically, preferably in an amount that is effective against the mentioned diseases, to a warm-blooded animal, e.g. man, requiring such treatment, preferably in the form of pharmaceutical compositions. The dose of the active ingredient depends on the species of the warm-blooded animal to be treated, its body weight, its age and individual status, individual pharmacokinetic circumstances, the disease to be treated and the application route. Preferably, for a body weight of approximately 70 kg a daily dose of from 0.001mg to 1000 mg, e.g. from approximately 0.01 mg to approximately 100 mg, preferably from approximately 0.05 mg to approximately 50 mg, of the active ingredient is administered.

The following Examples serve to illustrate the present invention, but should not be construed as a limitation thereof. The invention particularly relates to the specific embodiments (e.g. the proteins, nucleic acids, methods for the preparation, assays and uses thereof) as described in these Examples.

Abbreviations used herein have the following meaning:
aa = amino acid(s); bp: base pairs; BSA: bovine serum albumin; DTT: dithiothreitol; FCS: fetal calf serum; MALDI-TOF MS: Matrix Associated Laser Desorption Time-of Flight Mass Spectrum; ORF: open reading frame; PSA: puromycin-sensitive aminopeptidase; PBS: phosphate buffered saline; RT: room temperature; SDS-PAGE: sodium dodecyl sulfate - polyacrylamide gel electrophoresis; VSV: vesicular stomatitis virus

Example 1: Human Puromycin-Sensitive Aminopeptidases

Methods

Cloning procedures: 5×10^5 plaques of a human fetal brain ZAP II cDNA library (Stratagene; Catalogue No. 936206) are screened in duplicates with randomly primed radiolabeled POU domain DNA fragments of the oct-2 cDNA (bp 586-1077, Mueller, M.M. et al. (1988) Nature 336:544-551) and the oct-6 cDNA (bp 706-1232, Suzuki et al. (1990) Embo J. 9, 3723-3732).
The filters are washed in 2x SSC (150 mM sodium chloride; 15 mM sodium citrate) at 55°C and phages from plaques that give rise to identical signals on two filters are purified by another round of plating and screening and are then subcloned into Bluescript plasmid pBSII SK- (Stratagene). Sequence analysis is performed using a commercial kit (Sequenase-kit; USB) with "universal" and "reversed" primers matching to the flanks of the plasmid (pBSII SK-) polylinker. In four clones, no sequences at either end of the cDNAs are found related to known POU domain cDNAs. These clones are further analyzed by dot blot analysis and by in vitro transcription/translation followed by an SDS gel analysis. Dot blot analysis is performed with radiolabeled DNA probes of the human Oct-1 (Sturm, R.A. et al. (1988) Genes Dev. 2:1582-1599), Oct-2 (Mueller et al., supra), Oct-6 (Suzuki et al., supra), Brn-1, Brn-2, also referred to as N-Oct 3 (Schreiber, E. et al. (1993) Nucl. Ac. Res: 21, 253-258) and Brn4 cDNAs, using non-conserved regions of the POU cDNAs. Brn-1, Brn-2 and Brn-4 cDNAs are cloned from a human fetal brain cDNA library (He, X. et al. (1989), Nature 340: 35-42).One clone is identified encoding a protein that does not contain a POU domain. Clones with nested deletions of this cDNA (PSA-68 cDNA) are constructed. Both strands are sequenced using a commercial kit (Sequenase-kit; USB and "universal" and "reversed" primers. In a second round of screening, 1.0×10^6 plaques of the human fetal brain ZAP II cDNA library are screened using the radiolabelled, entire 3 kb cDNA of the murine PSA-99 enzyme (encoding an ORF of 920 aa; SEQ ID NO:7).

The screening procedure is as outlined above.

Northern blot analysis: Total RNA is isolated using the guanidine thiocyanate method. 20 µg are electrophoresed in 1% agarose/formaldehyde gels and transferred overnight to nitrocellulose membrane by blotting with 20x SSC. The RNA is immobilized by UV irradiation and the filters prehybridized for 1 h at 42°C in prehybridization solution (5x SSC, 1x Denhardt's solution, 50mM sodium phosphate pH 7.0, 1% SDS, 50% formamide, 200 µg/ml sonicated and denatured herring sperm DNA). Filters are hybridized overnight at 42°C in hybridization solution containing random primer labeled probes (8.3×10^3 Bq/ml). The following probes are used: probe 'all PSA' = 1124bp fragment of PflM1/StuI digest of PSA-99 cDNA (SEQ ID NO:1); probe 'PSA-99' = 409 bp fragment of HincII/Pflm1 digest of PSA-99 cDNA; probe 'PSA-93' = 243bp fragment of Pst1/Xho1 digest of PSA-93 cDNA (SEQ ID NO:3); probe 'PSA-68' = 562bp fragment of SalI/CelII digest of PSA-68 cDNA (SEQ ID NO:4). The blots are washed twice at RT in wash buffer 1 (1x SSC, 12 mM sodiumphospate pH 7.0, 0.5% SDS), once in wash buffer 1 at 65°C, and twice at 65°C in wash buffer 2 (0.2x SSC, 2.4mM sodium phosphate pH 7.0, 0.1% SDS). Filters are exposed to phosphor screens and scanned.

Protein extraction, in vitro transcription/translation and Western blot analysis

Transfected and untransfected cells in a 150 cm^2 flask are washed once in Hanks balanced salt solution (HBSS) and incubated for 5 min on ice in 2-4 ml ice cold cell lysis buffer (20mM Tris pH 7.5, 150mM NaCl, 1mM EDTA, 0.5% Triton X-100, 1mM PMSF, 1µM pepstatin A, 1µM leupeptin, 0.3µM aprotinin). The cells are scraped off and centrifuged for 30 min at 13'000 g /4°C to deplete the lysate of nuclei and particles. The extracts are frozen in liquid nitrogen and stored at - 70°C. For in vitro transcription/ translation, the TNT system from Promega and T7 RNA polymerase are used. Crude cell extracts are separated by SDS-PAGE on a 6% separating gel and transferred to nitrocellulose membranes. The membranes are blocked overnight at 4°C in 1% blocking solution (ECL kit; Boehringer Mannheim) containing 2% serum of the species the secondary antibody is produced in. The primary antibodies are diluted in 0.5% blocking solution; the peptide antibodies are used at a concentration of 5µg/ml; anti-VSV serum is diluted 1:500. Membranes are incubated 1h at 4°C with primary antibodies, washed twice 15min in PBS containing 0.1% Tween-20, once in 0.5% blocking solution and once in 1% blocking solution containing 2% serum. The secondary antibodies (goat anti-rabbit IgG, $F(ab')_2$ fragment; Sigma) are diluted according to the manufacturer's instructions in 0.5% blocking solution and the membranes are incubated for 1h at 4°C. After washing the membranes four times in PBS/0.1% Tween-20,

the bands are visualized using an enhanced chemiluminescence kit (Amersham). The membranes are exposed to X-ray films.

Immunoprecipitation: The entire procedure is performed at 4°C. 500 µl of crude cell lysate of transfected HeLa cells is incubated for 1h with 40 µl 50 % Protein G-sepharose (Pharmacia) in cell lysis buffer. The sepharose is spun down and the supernatant incubated with 1 µl anti-VSV antiserum. The sample is rotated for 2 h, then 20 µl 50% Protein G sepharose is added and the sample rotated again for 1 h. The sepharose is washed two times in cell lysis buffer without protease inhibitors, one time in cell lysis buffer without Triton X-100 and one time in 50 mM Tris, pH 6.8. To the sepharose pellet, 50 µl SDS loading dye (= 2 x SDS sample buffer; 125 mM Tris/Cl, pH 6.8, 4 % SDS, 5 % glycerol, 2 % β-mercaptoethanol) is added and the sample boiled for 5 min.

Transfection and staining of HeLa cells: The pSCT2 expression vectors used for transfecting HeLa cells are obtained from the laboratory of Prof. J.-C. Perriard (Swiss Federal Institute of Technology, Zürich). Plasmid pSCT2 is a modified version of pSCT1 containing a multiple cloning site (MCS).

The ORFs are amplified with the pBSII-SK- plasmids as templates isolated from the library using Taq DNA-polymerase. The PCR primers at the 3' end of the open reading frames are elongated with sequences encoding the VSV-tag, and both primers are further elongated with sequences suitable for ligation independent cloning.

HeLa cells are grown to 80% confluency in Dulbecco's modified Eagles medium containing penicillin / streptomycin and 10% FCS. At the day of transfection, cells are trypsinized, washed once in HBSS and resuspended at RT in 400µl electroporation buffer (25mM HEPES, 140mM NaCl, 0.75mM Na_2HPO_4 pH 7.1) at a density of 25 x 10^6 cells /ml. The cells are mixed with 80 µg plasmid DNA and incubated 5 min at RT. After electroporation, cells are left for 10 min at RT and resuspended in 10 ml medium. For immunofluorescence experiments, 30'000 cells are cultured on glass cover slips for two days; for protein extracts, the cells are cultured in a 150 cm^2 tissue culture flask. For immunofluorescent staining, the cells are fixed for 15 min in 3% paraformaldehyde/PBS; washed once with PBS, quenched for 5 min in 1mM glycine/PBS and permeabilized in 0.1% Triton X-100/PBS for 15 min. For the double staining with antibodies against the VSV-epitope and against α-tubulin, the cells are fixed in acetone/ethanol 1:1 at -20°C for 5 min. The cells are then washed twice in PBS and blocked in PBS/4% serum of the host animal the secondary antibody is produced in at 4°C for at least 1 h. The anti-VSV serum is diluted 1:500 in PBS/2% serum and incubated for 1 h at 4°C. The cells are then washed three times in PBS and blocked again in PBS/4%

serum. The secondary antibody is diluted according in PBS/2% serum and incubated for 1h at 4°C. The cells are again washed three times in PBS, dried and embedded in Mowiol. The stained cells are stored at 4°C.

Generation of anti-peptide antibodies: Two peptides are selected adjacent to the first two possible translation initiation sites in the PSA-99 cDNA: Peptide 1 has the amino acid sequence WLAAAAPSLARRLLFLGPPPPPLL (bp 276-345 in SEQ ID NO:1), and PEKRPFERLPADVSPINYSLCLKPDLLD (aa 2-29 in SEQ ID NO:2), respectively (the amino acid sequences are given in the single letter code). Both peptides are cross-linked to keyhole limpet hemocyanin, purified and combined with Freund's complete adjuvans. This cocktail is used for primary immunization of rabbits. The rabbits are boosted four times every second week. Both peptides give rise to comparable antisera in two different rabbits as judged from solid-phase ELISA.

In situ hybridization and histology: Brain tissue is collected from four patients undergoing neural surgery and from five autopsies performed less than 8 h after death. Samples included cerebral cortex of frontal, temporal, and occipital lobes, and the cerebellum. In addition, surgical specimens obtained from adult liver, kidney, and skeletal muscle are studied. All tissues are fixed in formaldehyde and embedded in paraffin. Tissue sections are cut at 5µm on slides coated with poly L-lysine.

For histology and immunohistochemistry, sections are stained with hematoxylin and eosin. The immunohistochemical studies are performed by applying the immuno alkaline-phosphatase (APAAP) method (Stein, H. et al., (1985) Lab. Invest. 52:676-683). Monoclonal mouse anti-human neurofilament protein antibodies are used as primary antibodies (Dako, Denmark).

For in situ hybridization, the single-stranded anti-sense and sense (negative control) RNA is generated using the pBSII SK- vector containing the PSA-68 cDNA. Single stranded sulphur-labeled RNA probes are generated by in-vitro transcription using SalI- or BamHI-linearized plasmid and T3 or T7 RNA-polymerase, respectively. In situ hybridization is performed as previously described (Milani, S. et al. (1994) Am. J. Path. 144:528-537). 5 µm sections are cut from paraffin-embedded tissue blocks, dewaxed, dehydrated, treated with 0.2 N HCl for 20 min, and enzymatically digested for 10 min in 0.125 mg/ml Pronase (Boehringer Mannheim), rinsed in 0.1 M glycine/PBS, and fixed for 20 min in 4% paraformaldehyde/PBS. After washing in PBS for 5 min, acetylation in acetic anhydride diluted 1:400 in 0.1 M triethanolamine, pH 8.0 for 10 min, and rinsing in PBS, the sections are dehydrated in graded ethanols and air-dried. Hybridization is carried out for 18 h at

50°C in 25 µl of a solution containing 8,3 x 10^3 Bq of sulphur-labeled RNA probe in 50 % formamide, 10 % dextran sulfate, 10mM DTT, 10mM Tris-HCl, pH 7.5; 10mM Na_3PO_4, 300mM NaCl, 5mM EDTA, 0.002 % Ficoll 400, 0.002 % polyvinylpyrrolidone/0.002 % bovine serum albumine/0.2 mg/ml yeast tRNA. After hybridization, the sections are rinsed for 4 h at 50°C in hybridization buffer and subsequently digested for 30 min at 37°C with 20 µg/ml RNaseA in 0.1 M Tris-HCl, pH 7.5/ 1mM EDTA, 0.5 mM NaCl. After a 30 min wash at 37°C in reaction buffer sections are further rinsed in 2x SSC and 0.1x SSC for 30 min each, dehydrated in graded ethanols and air dried. The slides are then dipped into Ilford G5 nuclear emulsion and after 2 h of drying exposed for 25 to 45 days at 4°C. The slides are developed, rinsed and fixed. After being washed in water, they are then counterstained with hematoxylin-eosin, dehydrated though graded ethanols and xylene and mounted under coverslips. Specimens containing more than four times background signals are scored positive. Matched sense slide probes are used as a negative control.

Results

Cloning and sequence analysis of three human PSA cDNAs: A human fetal brain cDNA library is screened with radiolabelled POU DNA fragments of the oct-2 and oct-6 cDNAs. The filters are washed under low stringency conditions in order to detect related proteins as well. During the characterization of cDNAs that reproducibly bind the probes, a clone is identified which, when transcribed and translated in vitro, produces a protein that does not bind to the octamer sequence (consensus sequence, ATGCAAAT) in a band shift assay. Furthermore, cDNA probes of the human POU-domain proteins Oct-1, Oct-2, Oct-6, Brn-1, Brn-2/N-Oct 3 and Brn-4 do not hybridize to this clone as determined by dot blot. Sequencing reveals a 3361 bp insert with a 280 bp region in the 5' untranslated region displaying 50% similarity to the oct-2 probe used for screening the library. The cDNA contains a 1800 bp open reading frame and an analysis of the deduced protein (PSA-68, aa 274-875 in SEQ ID NO:2) reveals that it contains at its N-terminus (aa 305 to 333 in SEQ ID NO:3) a zinc-binding motif (VVGHELAH..... LNEGF) highly conserved among the gluzincin aminopeptidases (Hooper, N. M. (1994), FEBS Lett. 354:1-6). When the human fetal brain cDNA library is rescreened with the radiolabeled PSA-99 cDNA isolated from a mouse neuroblastoma cDNA library (Example 2), two additional cDNAs with the same zinc-binding motif are identified. The three cDNAs are schematically shown in Fig.1. The predicted protein encoded by the PSA-99 cDNA contains a stretch of 10 amino acids (aa 2 to aa 11 in SEQ ID NO:1) identical to the N-terminal amino acid sequence of the puromycin-

sensitive aminopeptidase purified from rat brain (Dyer, S.H. et al. (1990), J. Neurochem. 54:547-54). This sequence is located immediately downstream of the second translation initiation site of the PSA-99. The PSA proteins encoded by the three cDNAs are named according to the molecular weights of the respective protein. From the cDNA library, 3 independent types of cDNAs encoding the PSA-99, one encoding the PSA-93 and two encoding the PSA-68 can be isolated. Sequence comparison reveals a region of 1863 bp shared by all cDNAs. The longest possible open reading frames encode proteins with predicted molecular weights of 103 kDa, 94 kDa, and 68 kDa, respectively. Only the translation initiation site of the PSA-68 cDNA is flanked by a Kozak-consensus like sequence (GCCA/G)CCATGG, the underlined nucleotides being key nucleotides for a strong translation initiation (Kozak, M. (1991) J. Biol. Chem. 266:19867-70). The second translation initiation sites of the PSA-99 and PSA-93 cDNAs harbor one of the two nucleotides associated with a strong initiation of translation and make them more likely to be used. The translation initiation site of the PSA-68 cDNA is sited within a region present in all three cDNAs. Four nucleotides upstream of the translation initiation codon, a 5-nucleotide insertion is found exclusively in this cDNA (SEQ ID NO:4).

Determination of the translation initiation site: For the determination of the translation initiation sites; the 3 predicted PSA ORFss are cloned into the eukaryotic expression vector pSCT2(+) downstream of a CMV promoter. In the case of the PSA-99 cDNA, an additional expression vector is constructed displaying only the second ATG start codon. The open reading frames are amplified by PCR and a sequence is added to the primer annealing at the 3' end of the open reading frame so that the proteins become tagged with a VSV epitope at their C-terminus (Kreis, T.E. (1986) EMBO J. 5:931-941). The primers are further elongated at their 5' end so that the fragments produced can be inserted into the expression vector by ligation independent cloning (Haun, R.S. et al. (1992) Biotechniques 13:515-518). The constructs are transcribed in vitro by T7 RNA polymerase and simultaneously translated in rabbit reticulocyte lysate. SDS-PAGE separation of the tagged proteins on a 7.5% gel and subsequent detection of the blotted proteins with a polyclonal rabbit serum raised against the VSV-epitope demonstrates that all constructs are translated in vitro, whereas the control reaction gives no signal. The proteins encoded by the two vectors containing the PSA-99 ORF have both a molecular weight of 99 kDa. Thus, irrespective of the presence or absence of the first ATG codon, the second one is used to initiate translation. The PSA-93 ORF produces two proteins with molecular weights of 93kDa and 86kDa. This corresponds to a translation initiation at the second and third ATG

start codon. Only the PSA-68 ORF is initiated at the first translation initiation site, producing a protein of 68 kDa.

To determine the translation initiation site used in vivo, HeLa cells are transfected with the same expression vectors used for the in vitro translation experiments. Two days after transfection of the cells, a crude protein extract is analyzed by immunoblotting. Only the PSA-99 can be detected with this method, suggesting that in HeLa cells, only the second translation initiation site of the PSA-99 open reading frame is used efficiently. However, upon immunoprecipitation, also PSA-93 and PSA-68 are detected. This suggests that also in vivo, translation can start at the second ATG codon in the PSA-93 ORF, or at the first ATG in the PSA-68 ORF. Therefore, these proteins are named PSA-99, PSA-93 and PSA-68, the numbers corresponding to the respective molecular weights.

To identify the ATG start codon used in PSA-99 transcripts that are expressed endogenously in non-transfected cells, antibodies are generated against the two N-terminal sequences of the proteins translated from either the first or second ATG start codon. Against the PSA-93 and PSA-68 proteins, no specific anti-peptide antibodies can be generated since their sequence is part of the PSA-99 protein. Protein extracts from the Lan1 human neuroblastoma cell line and from the human monocytic cell line U937 are analyzed. In Northern blots, Lan1 cells show the strongest expression of PSA transcripts, whereas U937 cells do not produce any message. The antibody directed against the N-terminal sequence following the second translation initiation site detects a protein of 99kDa in Lan1 cells, whereas the one directed against the sequence following the first translation initiation site cannot detect a protein with the molecular weight expected. No proteins with molecular weight of 99 or 103 kDa can be detected in extracts of U937 cells. These results demonstrate that also in Lan1 cells that express PSA endogenously, translation of the PSA-99 mRNA is initiated at the second ATG codon.

Northern blot analysis of PSA expression: To determine the tissue distribution of the different PSA mRNAs, three probes are constructed which hybridize exclusively to one mRNA species. An additional probe hybridizing to a region shared by all mRNAs is used to detect any transcripts containing PSA-related sequences. Analysis of RNA isolated from different human organs with the probe recognizing all transcripts reveals a major mRNA species of about 4.8 kb in all tissues examined (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas). Normalization of the absolute values to the β-actin signal demonstrates that PSA transcripts are most abundant in brain, expressed at similar levels in heart, placenta, kidney and pancreas, whereas lung and liver display the weakest signal.

The isoform specific probes for the PSA-99 and PSA-93 mRNAs produces too much background and cannot be used to analyze the tissue distribution of these transcripts, whereas the PSA-68 probe does not recognize a transcript.

In Northern blots of Lan1 cells, a single mRNA species migrating at about 4.8kb can be detected with the probe hybridizing to all three PSA-like mRNAs as well as with the probes hybridizing specifically to the PSA-99 and PSA-93 mRNAs. No PSA-68 specific mRNA can be detected. In parallel, RNA of U937 cells is analyzed but no signals are detected with the different PSA probes used. These results demonstrate that the two mRNAs encoding the proteins PSA-99 and PSA-93 migrate at similar positions. The intensity of the PSA-99 specific signal is stronger than the one obtained with the probe hybridizing to all PSA mRNAs. This can be explained by the crossreactivity of this probe with an mRNA migrating at a slighthly higher position. This crossreactivity is never observed with the probe hybridizing to all PSA transcripts and is therefore not produced by an additional PSA transcript. The PSA-68 mRNA cannot be detected in Northern blots of Lan1 cells indicating that this method is either not sensitive enough or that the Lan1 cells do not transcribe the message.

Intracellular localization of the PSA proteins: For the intracellular localization of the three PSA proteins, HeLa cells are transfected with the same expression vectors used to determine the translation initiation sites. Two days after transfection, the cells are fixed and stained with a polyclonal antiserum directed against the VSV-epitope. Upon visualization by a FITC-labeled secondary antibody, all three PSA proteins can be detected by confocal microscopy in a subset of cells, whereas untransfected cells produce only a faint background staining. The frequency of cells staining positive for the VSV-tagged PSA proteins is highest in PSA-99 transfected cultures. The strongest expression is observed in cells transfected with the PSA-99 construct containing only the second translation initiation codon, lacking the GC-rich 5' untranslated region. In PSA-93 and PSA-68 transfected cells, a minor proportion of cells express the transfected protein. All VSV-tagged PSA proteins can be localized in the cytoplasm, where they accumulate in a perinuclear region. No proteins can be detected in the nuclei. Only cells expressing the proteins at high levels display a faint nuclear staining. The cytoplasmic localization of the proteins is not uniform, since a fibrillary pattern can be observed in a subset of cells. The intracellular components with which PSA proteins interact partially co-localize with α-tubulin. Extracting the cells prior to fixation results in a loss of the immunoreactivity . This indicates that the PSA-proteins are not tightly associated with cytoskeletal proteins or other subcellular structures. The proteins

remain evenly distributed also in mitotic cells and no association with mitotic spindles is recognized.

In situ hybridization: Lan1 neuroblastoma cells harbor by far the most PSA transcripts of all types of cells examined including glioma cell lines. To ascertain the expression of PSA proteins in neurons, in situ hybridization studies are performed on adult human brain sections. As probe, the entire PSA-68 cDNA (SEQ ID NO:4) that is able to recognize all PSA isoforms is used. An intense expression of PSA transcripts is found in the perikaryon of cortical ganglion cells, which are strongly labeled by antibodies reacting with neurofilament proteins. In contrast, astrocytes and oligodendrocytes do not express detectable levels of PSA mRNA. In the cerebellum, a strong expression of PSA transcripts is detected in granule neurons, whereas Purkinjie cells stain less positive for PSA mRNA. Only background labeling is obtained in control hybridizations of the cortex as well as of the cerebellum using a sense probe. No PSA transcripts can be observed in either liver, kidney or skeletal muscle.

Example 2: Murine Puromycin-Sensitive Aminopeptidase

Methods

Cloning procedures: A cDNA library from the murine neuroblastoma cell line Neuro2A (American Type Culture Colletction (ATCC) accession no. CCL 131) is constructed using a λ zap cDNA synthesis kit from Stratagene. Of this library, 1.5×10^6 plaques are screened at low stringency using an NcoI/SpeI fragment (0.7 kb) of a human metallopeptidase cDNA (SEQ ID NO:1, bp 1223-1914).

Hybridization is performed at 42°C overnight in 5x sodium salt citrate buffer (SSC) containing 30 % formamide, 1x Denhardt's, 0.1 % sodium dodecyl sulfate, 50 mM sodium phosphate buffer pH 7.0, 0.2 mg/ml hering sperm DNA and 0.5×10^6 cpm/ml of ^{32}P-labelled probe, and followed by washing in 1x SSC at 42°C.

The inserts of isolated positive plaques are in-vivo excised. After generating exonuclease III deletions (Stratagene manual) from the largest of these clones, overlapping sequences of both DNA strands are obtained using a Sequenase kit (USB, Cleveland).

Northern blot hybridization: Mouse multiple tissue Northern blots are purchased from Clontech (Paolo Alto, CA). A PSA probe is synthesized from the full length mouse cDNA by random primer labelling and hybridized at 42°C overnight in 5x SSC containing 50% formamide, 50 mM sodium phosphate buffer pH 7.0, 1x Denhardt solution and 1% SDS.

Filters are washed twice at 65°C in 2x SSC for 5 min and twice at 62°C in 0.2x SSC containing 0.1% SDS. Filters are exposed to X-ray films for 2 days at -70°C.

Transient expression of PSA in COS-7 cells: The PSA cDNA is cloned into plasmid pSVL (Pharmacia) in two steps. First, the blunted NcoI/StuI fragment of the murine PSA cDNA (bp 1058-2868 in SEQ ID NO:7) is subcloned into the Sma I site of pSVL (Pharmacia). The resulting plasmid containing the 3' end of PSA is cut with XbaI and ligated with the XbaI fragment (5' end) of the PSA cDNA. For transfection experiments, COS-7 cells are grown to confluency in Dulbecco's modified Eagles medium containing 10% FCS and passaged 1:4. The following day, 3.9×10^6 cells are resuspended in RPMI medium containing 10 mM dextrose and 0.1 mM DTT and are mixed with 5-7.5 µg of plasmid DNA. Following electroporation, the cells are incubated in culture medium for 24 h, washed twice with HBSS, and grown for another 48-72 h in fresh medium to prepare cell extracts.

Aminopeptidase activity assay: Subconfluent cell monolayers are washed twice with PBS and then incubated in PBS containing 0.5 mM EDTA to bring the cells into suspension. Floating cells are collected, washed once in PBS and then incubated on ice for 10 min in 100 µl 10 mM Tris.HCl, pH 7.4. To obtain cell-free extracts, the cells are homogenized by ultrasonication, and the resulting lysates are centrifuged for 30 min with 13'000 g at 4°C. Supernatant is collected, and protein concentrations are determined using the BioRad protein assay and BSA as standard. Aliquots of the extracts corresponding to 5-20 µg protein are diluted to 250 µl with 10 mM Tris.HCl, pH 7.4, and reactions are initiated by adding an equal volume of 100 mM Tris-HCl pH 7.4 containing 4 mM amino acyl p-nitroanilide (Sigma), 0.1 mg/ml BSA and 1 mM DTT. After incubation at 37°C for 15 min, reactions are stopped by adding 500 µl of 0.1 M sodium acetate, pH 4, and absorbance of the liberated p-nitroaniline (p-NA) is measured spectrophotometrically at 405 nm. Activities are calculated assuming that 1U of enzyme liberates pNA at a rate of 1 nmol min^{-1} ($\varepsilon = 9500\ l \times mol^{-1} \times cm^{-1}$).

Western blot analysis: Crude cell extracts are separated by SDS-PAGE (20 µg protein per lane) on a 7.5% separating gel and transferred to nitrocellulose membranes. The membranes are blocked overnight at 4°C in 10 mM Tris.HCl, pH 7.5, containing 150 mM NaCl, 0.1% Tween-20, 5% skim milk (Gibco BRL), 2% BSA and 1% rabbit serum (Sigma).In the same buffer, goat anti-rat PSA antiserum is diluted 1:500 and incubated overnight at 4°C. Subsequently, filters are washed 3x in PBS containing 0.1% BSA (w/v) and 0.05% Tween-20 (v/v) and incubated for 1 h with biotinylated rabbit anti-goat IgG (DAKO, Glostrup,

DK) diluted 1:104 in blocking solution containing 0.5% mouse serum. After washing the filters 3x with PBS, bands are visualized using the vectastain ABC-AP kit from Vector Laboratories (Burlingame, CA).

Immunocytochemistry: 2.5 x 10^4 cells are plated per well. After 24 h, cells are washed in PBS and fixed for 15 min in a mixture of acetone and ethanol (1:1) which is pre-cooled to -20°C. After rehydrating the cells in PBS for 5 min, the slides are transferred to a moist chamber and incubated for 30 min in blocking solution consisting of PBS, 1% BSA (w/v) and 2% rat serum (v/v) (Sigma). Goat anti-rat PSA IgG is diluted in blocking solution to a concentration of 40 µg/ml and pre-absorbed for 1 h either with 10 µg/ml rat AP-N or rat PSA (Dyer, S.H. et al. (1990) J. Neurochem. 54, 547-554). Subsequently, cells are incubated either with untreated or pre-absorbed goat anti-rat PSA or with pre-immune goat IgG (Sigma). After 45 min, cells are washed 3x in PBS and then incubated for 30 min with a sheep anti-goat Fab fragment coupled to alkaline phosphatase (Boehringer Mannheim) which is diluted 1:80 in blocking solution. Alkaline phosphatase reaction is developed for 20-30 min according to the manufacturer's instructions. To stop the staining reaction, cells are washed with PBS and mounted in mowiol. Immunocytochemical analysis of detergent-extracted cells is performed as above, except that cells are extracted with detergent and fixed in PBS containing 3.7% formaldehyde (w/v) prior to staining (Deery, W.J. et al. (1984) J. Cell Biol. 98, 904-910). Briefly, cells are washed for 10 sec in 0.08M PIPES, pH 6.9, containing 10 mM EGTA, 1 mM $MgCl_2$, 0.1 mM GTP and 4% polyethylene glycol. Subsequently, the cells are transferred for 1.5 min to the same buffer containing 0.5% Triton X-100, followed by a 30 sec wash in extraction buffer and fixation for 20 min at 25°C.

Cell cycle analysis: PSA-transfected COS-7 cells are left untreated or are treated with bestatin (100 µg/ml), puromycin (5 µM), or with VP16 (80 µg/ml) for 14 h at 37°C Subsequently, cells are trypsinized, fixed with 4% formaldehyde in PBS for 10 min on ice, lysed with 0.1% triton-X100 in PBS for 10 min on ice, and stained with propidium iodide (50 µg/ml) (Nicoletti, I. et al. (1991) J. Immunol. Meth. 139, 271-279. Cells are analyzed by flow cytometry.

Quantification of the synthesis of DNA and protein: To assess the effect of various aminopeptidase inhibitors on cell proliferation, PSA-transfected COS-7 cells are plated in 96-well microtiter plates at a density of 5 x 10^3 cells/well, each well containing 200 µl medium with or without aminopeptidase inhibitors. After incubating the cells for 24 h at 37°C, they are pulsed for another 16 h with ^{3}H-thymidine (1 µCi/well), and the incorporated

radioactivity is measured using a liquid scintillation counter. Values represent means of triplicate measurements. To quantitate protein synthesis, COS-7 cells are washed twice with PBS, resuspended at a density of 10^6 ml^{-1} in leucine-free RPMI medium containing 5% FCS, and mixed in aliquots of 0.5 ml with equal volumes of the same medium containing puromycin or cycloheximide at various concentrations. After pre-incubating these suspensions at 37°C for 45 min, 5 µCi [^{3}H]-leucine (144 Ci/mmol; NEN, Boston, MA) is added to each sample for 30 min. Incorporated radioactivity is precipitated by adding 1ml of ice-cold 10% TCA followed by incubation on ice for 30 min. Precipitates are collected on filters and washed 3x with 3 ml 5% TCA. After air-drying the filters, bound radioactivity is quantitated by scintillation counting. Values are corrected by subtracting background (7-8 x 10^3 cpm) as determined in the absence of cells. Values represent means of duplicate measurements. Stock solutions of bestatin (25 mg/ml), puromycin (25 mM) and cycloheximide (10 mg/ml) are prepared in de-ionized water. All inhibitors are purchased from Sigma.

Analysis of DNA fragmentation: After incubating PSA-transfected COS cells for 12 to 40 h in the presence or absence of aminopeptidase inhibitors, cells are permeabilized with detergent as described previously (Nicoletti, I. et al. (1991) J. Immunol. Meth. 139, 271-279) to extract fragmented but not intact DNA. Such cell extracts are extracted with phenol, and subsequently, DNA is precipitated with ethanol and analyzed on 2% agarose gels after incubating the samples for 30 min with RNase A (1 µg/ml). In situ DNA end-labelling of COS cells is performed in microtiter plates using terminal transferase to incorporate biotinylated deoxyuridine triphosphate (50 µM), and streptavidine-alkaline phosphatase for detecting incorporation.

Results

Cloning and sequence analysis of murine PSA: A fragment of the human cDNA encoding PSA-99 is used to screen a cDNA library derived from the mouse neuroblastoma cell line Neuro2A. Five clones are isolated, one being derived most likely from an incompletely spliced mRNA. All of the four remaining clones contain inserts of similar size (3 kb) and encode an open reading frame of 920 amino acids (SEQ ID NOs:5 and 6). The deduced protein sequence is 27-40% identical to that of several known aminopeptidases (YSCII (Genbank accession no. X63998), yeast AAP1 (Genbank accession no. L 12542), rat AP-N (Genbank accession no. M26710), lacto Ala-AP (Genbank accession no. Z21701) human

AP-A (Genbank accession no. L14721), BP1/6C3 Genbank accession no. M29961). The similarity is most significant in a core region (aa 150 to 510 in SEQ ID NO:6) containing the motif HEXXH-$(X)_{18}$-E at aa 353-376 in SEQ ID NO:6. In the ORF, two potential translational start sites are identified at nucleotide positions 106 and 241, respectively, the former being immediately preceded by a stop codon (SEQ ID NO:5). Translational start at the first AUG is expected to give rise to a protein containing a hydrophobic N- terminal sequence of 26 residues which is interrupted after 10 amino acids by two arginines. Separated from these two arginines by a spacer of 19 amino acids, a stretch of five additional basic residues is located further downstream. Together, these basic residues form a potential bipartite nuclear localization signal (NLS). Interestingly, four out of five aa at positions 727-731 are also basic in nature and thus may represent a second NLS. In addition to these two NLS, one potential complementary NLS is identified at aa positions 111-115 consisting of a stretch of negatively charged residues. Furthermore, nine potential phosphorylation sites for casein kinase II and one for cAMP/cGMP-dependent kinase are identified, and two potential N- glycosylation sites are at positions 63 and 649, respectively (SEQ ID NO:6). Further analyses of the predicted amino acid sequence reveal that aa residues 269-285 (termed PSA I) and 683-701 (PSA II) are 72% and 40% similar, respectively, to the proteasome motif described by Zwickl et al. ((1992) Biochem. 31, 964-972). In addition, these sequences are found to resemble also the microtubule binding sites in *tau* protein and two other microtubule binding proteins, MAP-2 and MAP-4 (Lee, G. et al. (1988) Science 239, 285-288; Lewis, S.A. et al. (1988) Science 242, 936-939; Goedert, M. et al. (1989) EMBO J. 8, 393-39947; Aizawa, H. et al. (1989) J. Biol. Chem. 264, 5885-5890). Within PSA I and II, the only residues which are not similar to either the proteasome motif or the microtubule binding sites or to both are the two glutamates and the second proline residue within PSAII. PSA I resembles more the proteasome motif than does PSA II, whereas PSA II C-terminally contains the residues ProGlyGluGly which alignes better to microtubule binding sites than do the corresponding residues of PSA I. Given these similarities with microtubule binding sites, PSA I and PSA II are candidates to mediate the observed association of PSA with microtubules. This may be verified by expressing an epitope-tagged PSA and monitoring its cellular localization after disruption of PSA I and II.The presence of the proteasome-like motifs within the PSA sequence may indicate that PSA and proteasomes functionally interact. If this motif serves to provide the individual subunits with the capacity to assemble the complex, PSA may interact even physically with proteasomes. Another interesting

possibility is that PSA via PSA I and II can bind to both microtubules and 26S proteasomes thereby linking them to each other.

At aa positions 47-64 of the ORF the residues are almost identical to the N-terminal sequence of the purified rat puromycin-sensitive aminopeptidase (Dyer, S.H. et al. (1990) J. Neurochem. 54, 547-554).

Western blot analysis of PSA-transfected COS cells: To further substantiate that the present cDNA encodes mouse PSA, it is transfected into COS-7 cells by electroporation and expressed under the control of an SV40 promoter using the expression vector pSVL. Crude extracts of cells transfected either with pSVL-PSA or with vector alone are subjected to Western blot analysis. Using an antiserum against purified rat PSA (Dyer et al., supra), a 100 kDa band is identified in PSA-transfected cells and in control extracts. The intensity of the band is clearly higher in the PSA-transfectants as compared to control extracts, indicating its induction. This is consistent with data obtained by Northern blot analysis showing that PSA mRNA is expressed in control cells and strongly induced in PSA-transfected cells.

Induction of aminopeptidase activity in transfected COS cells To prove that the cDNA of SEQ ID NO:5 encodes for a peptidase, crude cell extracts of transfected COS cells are assayed for aminopeptidase activity using various synthetic amino acyl p-nitroanilides (p-NA) as substrates (amino = Lys, Met, Leu, Phe, Ala). Depending on the nature of the amino acyl residue, aminopeptidase activity is induced 10-20 fold in crude extracts of transfected cells as compared to mock transfected cells. In contrast, no significant induction of amino-peptidase activity is detected in the culture medium. Of the substrates examined, methionyl- and leucyl-pNA are most efficiently metabolized followed by lysyl- and alanyl-pNA. Pre-incubating COS cell extracts either with puromycin or with PSA antiserum or with the aminopeptidase inhibitor bestatin resulted in profound, dose-dependent inhibition of Leu-pNA peptidase activity. Inhibition is observed also in the presence of metal chelators such as EDTA and phenanthroline. In contrast, pepstatin, leupeptin and phosphoramidon have only minor or no significant inhibitory effects.

Tissue distribution of PSA: A multiple tissue Northern blot is hybridized with a PSA cDNA probe to identify the organs expressing PSA. A single PSA transcript of 4.5 kb is detectable in all organs examined (heart, brain, spleen, lung, liver, skeletal muscle, kidney, testis) suggesting that its expression is widely distributed. PSA mRNA is more abundant in the brain than in all other organs examined.

Subcellular localization of PSA: The hypothesis that PSA is involved in neuropeptide metabolism at postsynaptic membranes has been questioned since PSA has been shown to occur predominantly in a soluble cytoplasmic form. Only in the brain, a significant fraction of the protein is associated with membranes, and cell fractionation studies indicated that also the membrane-associated PSA is mainly an intracellular protein (Dyer et al., supra). Therefore, determining the subcellular sites of PSA expression should be helpful in understanding its functions. Using the IgG fraction of an antiserum against purified rat PSA, immunocytochemistry is performed in COS cells transfected either with the PSA expression vector or with the vector alone. PSA- transfected cells stain strongly positive. The staining is most concentrated in a perinuclear region where the nuclei usually are flattened. Such flattening of the nucleus defines a cellular polarity and indicates the site where the cytoplasmic microtubule complex (CMTC) originates and where the Golgi complex is anchored to the cytoskeleton. Transient transfection with PSA significantly increases the intensity of staining in this perinuclear region as compared to cells which resist transfection, or as compared to control-transfectants. Besides the CMTC/Golgi compartment, cellular processes frequently are stained. In mitotic cells the spindle apparatus also stains positive for PSA , and this staining is resistant to extraction of the cells with detergent,when performed in a microtubule- stabilizing buffer according to the protocol of Deery et al. ((1984) J. Cell Biol. 98, 904-910). Already during prophase PSA associates with the microtubules of arising asters and with the microtubule organizing centers. Some PSA-transfected cells also exhibit nuclear staining which sometimes is concentrated in large vesicular inclusions but usually is distributed throughout the nucleus. In contrast, no profound nuclear staining is observed in control- or non-transfected cells. The pattern of staining is specific. The finding that in COS cells the subcellular localization of PSA is heterogeneous leads to the hypothesis that it may be regulated in a cell cycle- specific manner. To further evaluate this possibility, endogenous PSA is localized within cells of the non-transformed murine fibroblast cell line Swiss 3T3. Like all other cell lines examined, 3T3 fibroblasts express PSA mRNA. 3T3 cells exhibit similar staining patterns as observed in untransfected COS cells, a difference being the nuclear staining observed in a subpopulation of 3T3 cells. Such nuclear staining appears to be associated with cells that are in prophase of the cell cycle. During metaphase, PSA staining is associated with the spindle apparatus, and it becomes concentrated around the chromosomes during anaphase. Most intense staining is observed in the newly formed nuclei and in the cytoplasm during telophase. Like in COS cells, also in 3T3 cells the microtubule-associated

staining resists extraction with detergent. Taken together, the data support the conclusion that PSA binds specifically to mitotic spindles and may play a role in proteolysis during mitosis.

Inhibitors of PSA arrest the cell cycle during G2/M-phase: To examine whether PSA activity may be essential during mitosis, the DNA content of COS cells is assessed by flow cytometry after treating the cells for 14 h with either puromycin or the aminopeptidase inhibitor bestatin, or with the etoposide VP16 which reacts with topoisomerase II and is known to induce a G2 block (Barry, M.A. et al. (1993) Cancer Res. 53, 2349-235). Both puromycin and bestatin lead to a significant accumulation of cells in G2/M-phase, the effect being comparable to or even exceeding that of VP16, respectively. This suggests that puromycin and bestatin inhibit the cell cycle during G2- or M-phase, or both.

Inhibition of proliferation: Since puromycin, besides of inhibiting PSA, is known also to interfere with protein synthesis, its effect is compared to those of cycloheximide, another inhibitor of protein synthesis, and of bestatin, using a DNA synthesis assay as a measure for cell proliferation. Both puromycin and cycloheximide as well as bestatin dose-dependently inhibit proliferation. However, unlike cycloheximide, puromycin significantly inhibits proliferation even at concentrations that do not detectably inhibit protein synthesis (< 1 µM), and the ID_{50} of puromycin is comparable to that required for half maximal inhibition of PSA activity (1 µM). Cells which are treated with puromycin or bestatin show the morphological characteristics of apoptosis, such as chromatin condensation around the margin of the nuclei, membrane blebbing and DNA fragmentation. Fragmentation of DNA is assessed by in situ DNA end-labelling in COS cells which are treated with bestatin (100 µg/ml) or puromycin (5 µM); DNA breaks are detected in about 76 % and about 81 % of the cells, respectively. This contrasts cycloheximide (17.8 µM) which ultimately leads to cell death without inducing more than 3% of the cells to undergo DNA fragmentation. These data are confirmed by gel electrophoretic analysis showing significant DNA fragmentation in puromycin- and bestatin-treated cells, but not in cells exposed to cycloheximide. These findings indicate that PSA inhibitors can induce cells to die via apoptosis.

Example 3: Antisense oligonucleotides

The following oligonucleotide analogues are obtained according to the following procedure:

The oligonucleotide derivatives of examples 1 to 15 and reference example 16 are synthesized on an Applied Biosystems 392 DNA-RNA Synthesizer (Applied Biosystems Inc., Foster City, California, USA) using standard cyanoethyl phosphoramidite chemistry without removal of the terminal dimethoxytrityl group. Controlled pore glass is used as the carrier material (Applied Biosystems Inc., Foster City, USA). For phosphorothioate oligonucleotides, the standard oxidation bottle is replaced by a 0.1 M solution of diisopropoxy-thiophoshoric acid disulfide in pyridine/acetonitrile 1 : 3 (v/v) at ambient temperature for the stepwise thiation of the phosphite linkages. The thiation step is followed by the capping step (acetic anhydride/2,6-lutidine/N-methylimidazole 12%/12%/4% (v/v/v) in tetrahydrofurane). The resulting crude oligonucleotide is deprotected using 33% aqueous ammonium hydroxide at 55 °C overnight. Subsequent purification involves reverse phase HPLC on a Waters HPLC system using a ®Nucleosil C_{18} column (10 µm mean bead diameter, silicagel derivatized with octadecyl silanes, obtainable from Macherey & Nagel, Düren, FRG) ((eluent: 0.05 M triethylammonium acetate, pH = 7.0, containing 10 vol-% acetonitrile, increasing to 45 vol-% within 50 min; length of column: 250 mm; diameter of column: 20 mm; flow rate: 15 ml/min; detection: UV absorption at 254 nm). The 5'-terminal dimethoxytrityl group is then removed by treatment with 80% acetic acid, followed by extraction with diethyl ether. The obtained oligonucleotide is dialysed using a regenerated cellulose dialysis membrane with a molecular weight cut-off 1.000 (®Spectra/Por Multiple Dialyzer; Spectrum Medical Industries, Inc., Los Angeles, USA) against 100 mM NaCl (once) and water (twice) and finally lyophilized. The correct molecular weight is confirmed by MALDI-TOF Mass Spectroscopy.

The following examplary oligonucleotide derivatives are obtained: All examples are phosphorothioate analogues of the corresponding natural oligo-2'-deoxyribonucleotides.

RNA target sequence (5'-3') (see SEQ ID NO:1)	SEQ ID NO:	Antisense oligonucleotide (5'-3')
8 - 27	7	TA ATG CAG AAG TAC ATC GG
71 - 90	8	GG GCT TGT CTA TCC CTC AC
131 - 150	9	TCC GCC ACA GAA CAT CTT AG

136 - 155	10	CAG GTT CCG CCA CAG AAC AT
181 - 200	11	TTT AGT CTT CCA GAA TCC AA
247 - 266	12	AGC GCG GGC CAC CGC CGG AG
264 - 283	13	GCC AGC CAC ATC CAC CGA GC
271 - 290	14	GGC AGC TGC CAG CCA CAT CC
400 - 419	15	CCT CTT CTC CGG CAT CGC GG
405 - 424	16	AAG GGC CTC TTC TCC GGC AT
501 - 520	17	GCG GCC TCC AGC TTG CCC TC
589 - 608	18	ATC TCC TTC TGG TGC ATA TG
951 - 970	19	CAC CTG GGC GGC GGC CTC CA
2966 - 2985	20	TGC TCT CAG CAT CTC GCT TT
3027 - 3046	21	CGC AAC CTC AGG ATT CAC AC
3149 - 3168	22	TTA ACT ACA TTC ATT GTC CA
3197 - 3216	23	TTT TCC TTT TTC AAT AGA AT
3261 - 3280	24	GCT CTA TTT TAT TAC TAT CA
3297-3316	25	ACT GAC AAG GCT TCA GAA AG
3381 - 3400	26	CCA AGA AGC CTC TAG TTT CC
3421 - 3440	27	TAG GCT CCT TGC AGA ACC CA
3507 - 3526	28	TTA TGC ACT GAC TCC AAC TA
3551 - 3570	29	TCC TGA TCA GTC CCC GTG CC
3556-3575	30	TAT CTT CCT GAT CAG TCC CC
3611 - 3630	31	GCA AGG AGG AAA ACT AAA CT

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: CIBA-GEIGY AG
(B) STREET: Klybeckstr. 141
(C) CITY: Basel
(E) COUNTRY: SCHWEIZ
(F) POSTAL CODE (ZIP): 4002
(G) TELEPHONE: +41 61 69 11 11
(H) TELEFAX: + 41 61 696 79 76
(I) TELEX: 962 991

(ii) TITLE OF INVENTION: Aminopeptidases

(iii) NUMBER OF SEQUENCES: 31

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3747 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:273..275
(D) OTHER INFORMATION:/function= "first translation initiation site"
/product= "PSA with molecular weight of 103 kDa for CDS extending from bp 273 to 3032"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:405..3032
(D) OTHER INFORMATION:/product= "Puromycin-sensitive aminopeptidase (PSA)-99"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAATTCCCCG ATGTACTTCT GCATTAAATT CCTTGCTTTC AGAATCATAA CCAGGGAATC 60

GTTTGGTACT GTGAGGGATA GACAAGCCCT CATTTCTGTA ATCCCTGCTA AGTGAGGTCA 120

AGGCTGACAT CTAAGATGTT CTGTGGCGGA ACCTGGCCCC CATCAGGGGC AACCTTTAAT 180

TTGGATTCTG GAAGACTAAA ACTTTGAGAT CTCTCCCCCG CCCCCCAGGC TCCCCCGGTA 240

GCTCTCCTCC GGCGGTGGCC CGCGCTCGGT GGATGTGGCT GGCAGCTGCC GCCCCCTCCC 300

TCGCTCGCCG CCTGCTTTTC CTCGGCCCTC CGCCTCCTCC CCTCCTCCTT CTCGTCTTCA 360

GCCGCTCCTC TCGCCGCCGC CTCCACAGCC TGGGCCTCGC CGCG ATG CCG GAG AAG 416
Met Pro Glu Lys
1

AGG CCC TTC GAG CGG CTG CCT GCC GAT GTC TCC CCC ATC AAC TAC AGC 464
Arg Pro Phe Glu Arg Leu Pro Ala Asp Val Ser Pro Ile Asn Tyr Ser
5 10 15 20

CTT TGC CTC AAG CCC GAC TTG CTG GAC TTC ACC TTC GAG GGC AAG CTG 512
Leu Cys Leu Lys Pro Asp Leu Leu Asp Phe Thr Phe Glu Gly Lys Leu
25 30 35

GAG GCC GCC GCC CAG GTG AGG CAG GCG ACT AAT CAG ATT GTG ATG AAT 560
Glu Ala Ala Ala Gln Val Arg Gln Ala Thr Asn Gln Ile Val Met Asn
40 45 50

TGT GCT GAT ATT GAT ATT ATT ACA GCT TCA TAT GCA CCA GAA GGA GAT 608
Cys Ala Asp Ile Asp Ile Ile Thr Ala Ser Tyr Ala Pro Glu Gly Asp
55 60 65

GAA GAA ATA CAT GCT ACA GGA TTT AAC TAT CAG AAT GAA GAT GAA AAA 656
Glu Glu Ile His Ala Thr Gly Phe Asn Tyr Gln Asn Glu Asp Glu Lys
70 75 80

GTC ACC TTG TCT TTC CCT AGT ACT CTG CAA ACA GGT ACG GGA ACC TTA 704
Val Thr Leu Ser Phe Pro Ser Thr Leu Gln Thr Gly Thr Gly Thr Leu
85 90 95 100

AAG ATA GAT TTT GTT GGA GAG CTG AAT GAC AAA ATG AAA GGT TTC TAT 752
Lys Ile Asp Phe Val Gly Glu Leu Asn Asp Lys Met Lys Gly Phe Tyr
105 110 115

AGA AGT AAA TAT ACT ACC CCT TCT GGA GAG GTG CGC TAT GCT GCT GTA 800
Arg Ser Lys Tyr Thr Thr Pro Ser Gly Glu Val Arg Tyr Ala Ala Val
120 125 130

ACA CAG TTT GAG GCT ACT GAT CCG CGA AGG GCT TTT CCT TGC TGG GAT 848
Thr Gln Phe Glu Ala Thr Asp Pro Arg Arg Ala Phe Pro Cys Trp Asp
135 140 145

GAG CCT GCT ATC AAA GCA ACT TTT GAT ATC TCA TTG GTT GTT CCT AAA 896
Glu Pro Ala Ile Lys Ala Thr Phe Asp Ile Ser Leu Val Val Pro Lys
150 155 160

GAC AGA GTA GCT TTA TCA AAC ATG AAT GTA ATT GAC CGG AAA CCA TAC 944
Asp Arg Val Ala Leu Ser Asn Met Asn Val Ile Asp Arg Lys Pro Tyr
165 170 175 180

CCT GAT GAT GAA AAT TTA GTG GAA GTG AAG TTT GCC CGC ACA CCT GTT 992
Pro Asp Asp Glu Asn Leu Val Glu Val Lys Phe Ala Arg Thr Pro Val
185 190 195

ATG TCT ACA TAT CTG GTG GCA TTT GTT GTG GGT GAA TAT GAC TTT GTA 1040
Met Ser Thr Tyr Leu Val Ala Phe Val Val Gly Glu Tyr Asp Phe Val
200 205 210

GAA ACA AGG TCA AAA GAT GGT GTG TGT GTC CGT GTT TAC ACT CCT GTT 1088
Glu Thr Arg Ser Lys Asp Gly Val Cys Val Arg Val Tyr Thr Pro Val
215 220 225

GGC AAA GCA GAG CAA GGA AAA TTT GCG TTA GAG GTT GCT GCT AAA ACC 1136
Gly Lys Ala Glu Gln Gly Lys Phe Ala Leu Glu Val Ala Ala Lys Thr
230 235 240

TTG CCT TTT TAT AAG GAC TAC TTC AAT GTT CCT TAT CCT CTA CCT AAA 1184
Leu Pro Phe Tyr Lys Asp Tyr Phe Asn Val Pro Tyr Pro Leu Pro Lys
245 250 255 260

ATT GAT CTC ATT GCT ATT GCA GAC TTT GCA GCT GGT GCC ATG GAG AAC 1232
Ile Asp Leu Ile Ala Ile Ala Asp Phe Ala Ala Gly Ala Met Glu Asn
265 270 275

TGG GGC CTT GTT ACT TAT AGG GAG ACT GCA TTG CTT ATT GAT CCA AAA 1280
Trp Gly Leu Val Thr Tyr Arg Glu Thr Ala Leu Leu Ile Asp Pro Lys
280 285 290

AAT TCC TGT TCT TCA TCC CGC CAG TGG GTT GCT CTG GTT GTG GGA CAT 1328
Asn Ser Cys Ser Ser Ser Arg Gln Trp Val Ala Leu Val Val Gly His
295 300 305

GAA CTC GCC CAT CAA TGG TTT GGA AAT CTT GTT ACT ATG GAA TGG TGG 1376

Glu Leu Ala His Gln Trp Phe Gly Asn Leu Val Thr Met Glu Trp Trp
310 315 320

ACT CAT CTT TGG TTA AAT GAA GGT TTT GCA TCC TGG ATT GAA TAT CTG 1424
Thr His Leu Trp Leu Asn Glu Gly Phe Ala Ser Trp Ile Glu Tyr Leu
325 330 335 340

TGT GTA GAC CAC TGC TTC CCA GAG TAT GAT ATT TGG ACT CAG TTT GTT 1472
Cys Val Asp His Cys Phe Pro Glu Tyr Asp Ile Trp Thr Gln Phe Val
345 350 355

TCT GCT GAT TAC ACC CGT GCC CAG GAG CTT GAC GCC TTA GAT AAC AGC 1520
Ser Ala Asp Tyr Thr Arg Ala Gln Glu Leu Asp Ala Leu Asp Asn Ser
360 365 370

CAT CCT ATT GAA GTC AGT GTG GGC CAT CCA TCT GAG GTT GAT GAG ATA 1568
His Pro Ile Glu Val Ser Val Gly His Pro Ser Glu Val Asp Glu Ile
375 380 385

TTT GAT GCT ATA TCA TAT AGC AAA GGT GCA TCT GTC ATC CGA ATG CTG 1616
Phe Asp Ala Ile Ser Tyr Ser Lys Gly Ala Ser Val Ile Arg Met Leu
390 395 400

CAT GAC TAC ATT GGG GAT AAG GAC TTT AAG AAA GGA ATG AAC ATG TAT 1664
His Asp Tyr Ile Gly Asp Lys Asp Phe Lys Lys Gly Met Asn Met Tyr
405 410 415 420

TTA ACC AAG TTC CAA CAA AAG AAT GCT GCC ACA GAG GAT CTC TGG GAA 1712
Leu Thr Lys Phe Gln Gln Lys Asn Ala Ala Thr Glu Asp Leu Trp Glu
425 430 435

AGT TTA GAA AAT GCT AGT GGT AAA CCT ATA GCA GCT GTG ATG AAT ACC 1760
Ser Leu Glu Asn Ala Ser Gly Lys Pro Ile Ala Ala Val Met Asn Thr
440 445 450

```
TGG ACC AAA CAA ATG GGA TTT CCC CTC ATT TAT GTG GAA GCT GAA CAG      1808
Trp Thr Lys Gln Met Gly Phe Pro Leu Ile Tyr Val Glu Ala Glu Gln
        455                 460                 465

GTA GAA GAT GAC AGA TTA TTG AGG TTG TCC CAA AAG AAG TTC TGT GCT      1856
Val Glu Asp Asp Arg Leu Leu Arg Leu Ser Gln Lys Lys Phe Cys Ala
    470                 475                 480

GGT GGG TCA TAT GTT GGT GAA GAT TGT CCC CAG TGG ATG GTC CCT ATC      1904
Gly Gly Ser Tyr Val Gly Glu Asp Cys Pro Gln Trp Met Val Pro Ile
485                 490                 495                 500

ACA ATC TCT ACT AGT GAA GAC CCC AAC CAG GCC AAA CTA AAA ATT CTA      1952
Thr Ile Ser Thr Ser Glu Asp Pro Asn Gln Ala Lys Leu Lys Ile Leu
                505                 510                 515

ATG GAC AAG CCA GAG ATG AAT GTG GTT TTG AAA AAT GTC AAA CCA GAC      2000
Met Asp Lys Pro Glu Met Asn Val Val Leu Lys Asn Val Lys Pro Asp
            520                 525                 530

CAA TGG GTG AAG TTA AAC TTA GGA ACA GTT GGG TTT TAT CGG ACC CAG      2048
Gln Trp Val Lys Leu Asn Leu Gly Thr Val Gly Phe Tyr Arg Thr Gln
        535                 540                 545

TAC AGC TCT GCC ATG CTG GAA AGT TTA TTA CCA GGC ATT CGT GAC CTT      2096
Tyr Ser Ser Ala Met Leu Glu Ser Leu Leu Pro Gly Ile Arg Asp Leu
    550                 555                 560

TCT CTG CCC CCT GTG GAT CGA CTT GGA TTA CAG AAT GAC CTC TTC TCC      2144
Ser Leu Pro Pro Val Asp Arg Leu Gly Leu Gln Asn Asp Leu Phe Ser
565                 570                 575                 580

TTG GCT CGA GCT GGA ATC ATT AGC ACT GTA GAG GTT CTA AAA GTC ATG      2192
Leu Ala Arg Ala Gly Ile Ile Ser Thr Val Glu Val Leu Lys Val Met
                585                 590                 595
```

GAG GCT TTT GTG AAT GAG CCC AAT TAT ACT GTA TGG AGC GAC CTG AGC 2240
Glu Ala Phe Val Asn Glu Pro Asn Tyr Thr Val Trp Ser Asp Leu Ser
600 605 610

TGT AAC CTG GGG ATT CTC TCA ACT CTC TTG TCC CAC ACA GAC TTC TAT 2288
Cys Asn Leu Gly Ile Leu Ser Thr Leu Leu Ser His Thr Asp Phe Tyr
615 620 625

GAG GAA ATC CAG GAG TTT GTG AAA GAT GTC TTT TCA CCT ATA GGG GAG 2336
Glu Glu Ile Gln Glu Phe Val Lys Asp Val Phe Ser Pro Ile Gly Glu
630 635 640

AGA CTG GGC TGG GAC CCC AAA CCT GGA GAA GGT CAT CTC GAT GCA CTC 2384
Arg Leu Gly Trp Asp Pro Lys Pro Gly Glu Gly His Leu Asp Ala Leu
645 650 655 660

CTG AGG GGC TTG GTT CTG GGA AAA CTA GGA AAA GCA GGA CAT AAG GCA 2432
Leu Arg Gly Leu Val Leu Gly Lys Leu Gly Lys Ala Gly His Lys Ala
665 670 675

ACG TTA GAA GAA GCC CGT CGT CGG TTT AAG GAC CAC GTG GAA GGA AAA 2480
Thr Leu Glu Glu Ala Arg Arg Arg Phe Lys Asp His Val Glu Gly Lys
680 685 690

CAG ATT CTC TCC GCT GAT CTG AGG AGT CCT GTC TAT CTG ACT GTT TTG 2528
Gln Ile Leu Ser Ala Asp Leu Arg Ser Pro Val Tyr Leu Thr Val Leu
695 700 705

AAG CAT GGT GAT GGC ACT ACT TTA GAT ATT ATG TTA AAA CTT CAT AAA 2576
Lys His Gly Asp Gly Thr Thr Leu Asp Ile Met Leu Lys Leu His Lys
710 715 720

CAA GCA GAT ATG CAA GAA GAG AAA AAC CGA ATC GAA AGA GTC CTT GGC 2624
Gln Ala Asp Met Gln Glu Glu Lys Asn Arg Ile Glu Arg Val Leu Gly
725 730 735 740

```
GCT ACT CTT TTG CCT GAC CTG ATT CAA AAA GTC CTC ACG TTT GCA CTT      2672
Ala Thr Leu Leu Pro Asp Leu Ile Gln Lys Val Leu Thr Phe Ala Leu
                745                 750                 755

TCA GAA GAG GTA CGT CCA CAG GAC ACT GTA TCG GTA ATT GGT GGA GTA      2720
Ser Glu Glu Val Arg Pro Gln Asp Thr Val Ser Val Ile Gly Gly Val
            760                 765                 770

GCT GGA GGC AGC AAG CAT GGT AGG AAA GCT GCT TGG AAA TTC ATA AAG      2768
Ala Gly Gly Ser Lys His Gly Arg Lys Ala Ala Trp Lys Phe Ile Lys
        775                 780                 785

GAC AAC TGG GAA GAA CTT TAT AAC CGA TAC CAG GGA GGA TTC TTA ATA      2816
Asp Asn Trp Glu Glu Leu Tyr Asn Arg Tyr Gln Gly Gly Phe Leu Ile
    790                 795                 800

TCC AGA CTA ATA AAG CTA TCA GTT GAG GGA TTT GCA GTT GAT AAA ATG      2864
Ser Arg Leu Ile Lys Leu Ser Val Glu Gly Phe Ala Val Asp Lys Met
805                 810                 815                 820

GCT GGA GAG GTT AAG GCT TTC TTC GAG AGT CAC CCA GCT CCT TCA GCT      2912
Ala Gly Glu Val Lys Ala Phe Phe Glu Ser His Pro Ala Pro Ser Ala
                825                 830                 835

GAG CGT ACC ATC CAG CAG TGT TGT GAA AAT ATT CTG CTG AAT GCT GCC      2960
Glu Arg Thr Ile Gln Gln Cys Cys Glu Asn Ile Leu Leu Asn Ala Ala
            840                 845                 850

TGG CTA AAG CGA GAT GCT GAG AGC ATC CAC CAG TAC CTC CTT CAG CGG      3008
Trp Leu Lys Arg Asp Ala Glu Ser Ile His Gln Tyr Leu Leu Gln Arg
        855                 860                 865

AAG GCC TCA CCA CCC ACA GTG TGA ATCCTGAGGT TGCGCCATTG GCGGTTCTGC     3062
Lys Ala Ser Pro Pro Thr Val
    870                 875

TCGTTCGCTG CAGGGATAAG GTGGAGCTAC CGAACAGCTG ATTCATATGC CAAGAATTTG    3122
```

GAGTCTTCTT TCAAACCAGT GGGGGTTGGA CAATGAATGT AGTTAACTGG TTCCTGCTCA 3182

CACTCCAGAA TTAAATTCTA TTGAAAAAGG AAAATCAGCA ATTCAGCAAA AAATAAATAA 3242

AAAATAAAAA TGTAAATATG ATAGTAATAA AATAGAGCAT AACGAAACTG TGAAACTTTC 3302

TGAAGCCTTG TCAGTGGTTA AAAGTATTTA ACACTCTACT GTTAATGACA GATGTTCTGT 3362

TTTTATAACC TACCAAAAGG AAACTAGAGG CTTCTTGGTG AAGAGCATTT TTGTGAAGTG 3422

GGTTCTGCAA GGAGCCTATA AAGCCAAGGG TGGTGTCCAT TTCTGGGAAT GGTTAAACAC 3482

AAAAGGCTGA TAGCTGGTAT CACATAGTTG GAGTCAGTGC ATAATTCCAA GTGGCTTTTT 3542

TTTTTTTTGG CACGGGGACT GATCAGGAAG ATATATTCCT GCATAACTCA ATCTGAACCA 3602

AGGATTGTAG TTTAGTTTTC CTCCTTGCCT TCCCTTCTGT GTGACCGACC CCTTGGCCAA 3662

AAAAAAAACA AAAAGCAAAA AACAAAAACC TACCCTGTTC TGGTTTTTTT CCTCCCTTTA 3722

GTTCCACCCC CAACCCCCGG AATTC 3747

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 875 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Pro Glu Lys Arg Pro Phe Glu Arg Leu Pro Ala Asp Val Ser Pro
1 5 10 15

Ile Asn Tyr Ser Leu Cys Leu Lys Pro Asp Leu Leu Asp Phe Thr Phe
20 25 30

Glu Gly Lys Leu Glu Ala Ala Ala Gln Val Arg Gln Ala Thr Asn Gln
35 40 45

Ile Val Met Asn Cys Ala Asp Ile Asp Ile Ile Thr Ala Ser Tyr Ala
50 55 60

Pro Glu Gly Asp Glu Glu Ile His Ala Thr Gly Phe Asn Tyr Gln Asn
65 70 75 80

Glu Asp Glu Lys Val Thr Leu Ser Phe Pro Ser Thr Leu Gln Thr Gly
85 90 95

Thr Gly Thr Leu Lys Ile Asp Phe Val Gly Glu Leu Asn Asp Lys Met
100 105 110

Lys Gly Phe Tyr Arg Ser Lys Tyr Thr Thr Pro Ser Gly Glu Val Arg
115 120 125

Tyr Ala Ala Val Thr Gln Phe Glu Ala Thr Asp Pro Arg Arg Ala Phe
130 135 140

Pro Cys Trp Asp Glu Pro Ala Ile Lys Ala Thr Phe Asp Ile Ser Leu
145 150 155 160

Val Val Pro Lys Asp Arg Val Ala Leu Ser Asn Met Asn Val Ile Asp
165 170 175

Arg Lys Pro Tyr Pro Asp Asp Glu Asn Leu Val Glu Val Lys Phe Ala
180 185 190

Arg Thr Pro Val Met Ser Thr Tyr Leu Val Ala Phe Val Val Gly Glu
195 200 205

Tyr Asp Phe Val Glu Thr Arg Ser Lys Asp Gly Val Cys Val Arg Val
210 215 220

Tyr Thr Pro Val Gly Lys Ala Glu Gln Gly Lys Phe Ala Leu Glu Val
225 230 235 240

Ala Ala Lys Thr Leu Pro Phe Tyr Lys Asp Tyr Phe Asn Val Pro Tyr
245 250 255

Pro Leu Pro Lys Ile Asp Leu Ile Ala Ile Ala Asp Phe Ala Ala Gly
260 265 270

Ala Met Glu Asn Trp Gly Leu Val Thr Tyr Arg Glu Thr Ala Leu Leu
275 280 285

Ile Asp Pro Lys Asn Ser Cys Ser Ser Ser Arg Gln Trp Val Ala Leu
290 295 300

Val Val Gly His Glu Leu Ala His Gln Trp Phe Gly Asn Leu Val Thr
305 310 315 320

Met Glu Trp Trp Thr His Leu Trp Leu Asn Glu Gly Phe Ala Ser Trp
325 330 335

Ile Glu Tyr Leu Cys Val Asp His Cys Phe Pro Glu Tyr Asp Ile Trp
340 345 350

Thr Gln Phe Val Ser Ala Asp Tyr Thr Arg Ala Gln Glu Leu Asp Ala
355 360 365

Leu Asp Asn Ser His Pro Ile Glu Val Ser Val Gly His Pro Ser Glu
370 375 380

Val Asp Glu Ile Phe Asp Ala Ile Ser Tyr Ser Lys Gly Ala Ser Val
385 390 395 400

Ile Arg Met Leu His Asp Tyr Ile Gly Asp Lys Asp Phe Lys Lys Gly
405 410 415

Met Asn Met Tyr Leu Thr Lys Phe Gln Gln Lys Asn Ala Ala Thr Glu
420 425 430

Asp Leu Trp Glu Ser Leu Glu Asn Ala Ser Gly Lys Pro Ile Ala Ala
435 440 445

Val Met Asn Thr Trp Thr Lys Gln Met Gly Phe Pro Leu Ile Tyr Val
450 455 460

Glu Ala Glu Gln Val Glu Asp Asp Arg Leu Leu Arg Leu Ser Gln Lys
465 470 475 480

Lys Phe Cys Ala Gly Gly Ser Tyr Val Gly Glu Asp Cys Pro Gln Trp
485 490 495

Met Val Pro Ile Thr Ile Ser Thr Ser Glu Asp Pro Asn Gln Ala Lys
500 505 510

Leu Lys Ile Leu Met Asp Lys Pro Glu Met Asn Val Val Leu Lys Asn
515 520 525

Val Lys Pro Asp Gln Trp Val Lys Leu Asn Leu Gly Thr Val Gly Phe
530 535 540

Tyr Arg Thr Gln Tyr Ser Ser Ala Met Leu Glu Ser Leu Leu Pro Gly
545 550 555 560

Ile Arg Asp Leu Ser Leu Pro Pro Val Asp Arg Leu Gly Leu Gln Asn
565 570 575

Asp Leu Phe Ser Leu Ala Arg Ala Gly Ile Ile Ser Thr Val Glu Val
580 585 590

Leu Lys Val Met Glu Ala Phe Val Asn Glu Pro Asn Tyr Thr Val Trp
595 600 605

Ser Asp Leu Ser Cys Asn Leu Gly Ile Leu Ser Thr Leu Leu Ser His
610 615 620

Thr Asp Phe Tyr Glu Glu Ile Gln Glu Phe Val Lys Asp Val Phe Ser
625 630 635 640

Pro Ile Gly Glu Arg Leu Gly Trp Asp Pro Lys Pro Gly Glu Gly His
645 650 655

Leu Asp Ala Leu Leu Arg Gly Leu Val Leu Gly Lys Leu Gly Lys Ala
660 665 670

Gly His Lys Ala Thr Leu Glu Glu Ala Arg Arg Arg Phe Lys Asp His
675 680 685

Val Glu Gly Lys Gln Ile Leu Ser Ala Asp Leu Arg Ser Pro Val Tyr
690 695 700

Leu Thr Val Leu Lys His Gly Asp Gly Thr Thr Leu Asp Ile Met Leu
705 710 715 720

Lys Leu His Lys Gln Ala Asp Met Gln Glu Glu Lys Asn Arg Ile Glu
725 730 735

Arg Val Leu Gly Ala Thr Leu Leu Pro Asp Leu Ile Gln Lys Val Leu
740 745 750

Thr Phe Ala Leu Ser Glu Glu Val Arg Pro Gln Asp Thr Val Ser Val
755 760 765

Ile Gly Gly Val Ala Gly Gly Ser Lys His Gly Arg Lys Ala Ala Trp
770 775 780

Lys Phe Ile Lys Asp Asn Trp Glu Glu Leu Tyr Asn Arg Tyr Gln Gly
785 790 795 800

Gly Phe Leu Ile Ser Arg Leu Ile Lys Leu Ser Val Glu Gly Phe Ala
805 810 815

Val Asp Lys Met Ala Gly Glu Val Lys Ala Phe Phe Glu Ser His Pro
820 825 830

Ala Pro Ser Ala Glu Arg Thr Ile Gln Gln Cys Cys Glu Asn Ile Leu
835 840 845

Leu Asn Ala Ala Trp Leu Lys Arg Asp Ala Glu Ser Ile His Gln Tyr
850 855 860

Leu Leu Gln Arg Lys Ala Ser Pro Pro Thr Val
865 870 875

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3153 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:64..2598
(D) OTHER INFORMATION:/product= "CDS for human PSA-93
(translation from 1. ATG)"

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(B) LOCATION:121..2598

(D) OTHER INFORMATION:/product= "CDS for human PSA-93, translation starting at 2. ATG"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GAATTCCAAA AAATAGCTGG CTGTGGTGGC GCATGACTGT GGTCCTAACT ACTCGGGAGG 60

CTTATGCAGG AGGATCATTT GAGCCCAGAA GGTGAGGCTG CACTGACTAA TCAGATTGTG 120

ATGAATTGTG CTGATATTGA TATTATTACA GCTTCATATG CACCAGAAGG AGATGAAGAA 180

ATACATGCTA CAGGATTTAA CTATCAGAAT GAAGATGAAA AAGTCACCTT GTCTTTCCCT 240

AGTACTCTGC AAACAGGTAC GGGAACCTTA AAGATAGATT TTGTTGGAGA GCTGAATGAC 300

AAAATGAAAG GTTTCTATAG AAGTAAATAT ACTACCCCTT CTGGAGAGGT GCGCTATGCT 360

GCTGTAACAC AGTTTGAGGC TACTGATCGC CGAAGGGCTT TTCCTTGCTG GGATGAGCCT 420

GCTATCAAAG CAACTTTTGA TATCTCATTG GTTGTTCCTA AAGACAGAGT AGCTTTATCA 480

AACATGAATG TAATTGACCG GAAACCATAC CCTGATGATG AAAATTTAGT GGAAGTGAAG 540

TTTGCCCGCA CACCTGTTAT GTCTACATAT CTGGTGGCAT TTGTTGTGGG TGAATATGAC 600

TTTGTAGAAA CAAGGTCAAA AGATGGTGTG TGTGTCCGTG TTTACACTCC TGTTGGCAAA 660

GCAGAGCAAG GAAAATTTGC GTTAGAGGTT GCTGCTAAAA CCTTGCCTTT TTATAAGGAC 720

TACTTCAATG TTCCTTATCC TCTACCTAAA ATTGATCTCA TTGCTATTGC AGACTTTGCA 780

GCTGGTGCCA TGGAGAACTG GGGCCTTGTT ACTTATAGGG AGACTGCATT GCTTATTGAT 840

CCAAAAAATT CCTGTTCTTC ATCCCGCCAG TGGGTTGCTC TGGTTGTGGG ACATGAACTC 900

GCCCATCAAT GGTTTGGAAA TCTTGTTACT ATGGAATGGT GGACTCATCT TTGGTTAAAT 960

GAAGGTTTTG CATCCTGGAT TGAATATCTG TGTGTAGACC ACTGCTTCCC AGAGTATGAT 1020

ATTTGGACTC AGTTTGTTTC TGCTGATTAC ACCCGTGCCC AGGAGCTTGA CGCCTTAGAT 1080

AACAGCCATC CTATTGAAGT CAGTGTGGGC CATCCATCTG AGGTTGATGA GATATTTGAT 1140

GCTATATCAT ATAGCAAAGG TGCATCTGTC ATCCGAATGC TGCATGACTA CATTGGGGAT 1200

AAGGACTTTA AGAAAGGAAT GAACATGTAT TTAACCAAGT TCCAACAAAA GAATGCTGCC 1260

ACAGAGGATC TCTGGGAAAG TTTAGAAAAT GCTAGTGGTA AACCTATAGC AGCTGTGATG 1320

AATACCTGGA CCAAACAAAT GGGATTTCCC CTCATTTATG TGGAAGCTGA ACAGGTAGAA 1380

GATGACAGAT TATTGAGGTT GTCCCAAAAG AAGTTCTGTG CTGGTGGGTC ATATGTTGGT 1440

GAAGATTGTC CCCAGTGGAT GGTCCCTATC ACAATCTCTA CTAGTGAAGA CCCCAACCAG 1500

GCCAAACTAA AAATTCTAAT GGACAAGCCA GAGATGAATG TGGTTTTGAA AAATGTCAAA 1560

CCAGACCAAT GGGTGAAGTT AAACTTAGGA ACAGTTGGGT TTTATCGGAC CCAGTACAGC 1620

TCTGCCATGC TGGAAAGTTT ATTACCAGGC ATTCGTGACC TTTCTCTGCC CCCTGTGGAT 1680

CGACTTGGAT TACAGAATGA CCTCTTCTCC TTGGCTCGAG CTGGAATCAT TAGCACTGTA 1740

GAGGTTCTAA AAGTCATGGA GGCTTTTGTG AATGAGCCCA ATTATACTGT ATGGAGCGAC 1800

CTGAGCTGTA ACCTGGGGAT TCTCTCAACT CTCTTGTCCC ACACAGACTT CTATGAGGAA 1860

ATCCAGGAGT TTGTGAAAGA TGTCTTTTCA CCTATAGGGG AGAGACTGGG CTGGGACCCC 1920

AAACCTGGAG AAGGTCATCT CGATGCACTC CTGAGGGGCT TGGTTCTGGG AAAACTAGGA 1980

AAAGCAGGAC ATAAGGCAAC GTTAGAAGAA GCCCGTCGTC GGTTTAAGGA CCACGTGGAA 2040

GGAAAACAGA TTCTCTCCGC TGATCTGAGG AGTCCTGTCT ATCTGACTGT TTTGAAGCAT 2100

GGTGATGGCA CTACTTTAGA TATTATGTTA AAACTTCATA AACAAGCAGA TATGCAAGAA 2160

GAGAAAAACC GAATCGAAAG AGTCCTTGGC GCTACTCTTT TGCCTGACCT GATTCAAAAA 2220

GTCCTCACGT TTGCACTTTC AGAAGAGGTA CGTCCACAGG ACACTGTATC GGTAATTGGT 2280

GGAGTAGCTG GAGGCAGCAA GCATGGTAGG AAAGCTGCTT GGAAATTCAT AAAGGACAAC 2340

TGGGAAGAAC TTTATAACCG ATACCAGGGA GGATTCTTAA TATCCAGACT AATAAAGCTA 2400

TCAGTTGAGG GATTTGCAGT TGATAAAATG GCTGGAGAGG TTAAGGCTTT CTTCGAGAGT 2460

CACCCAGCTC CTTCAGCTGA GCGTACCATC CAGCAGTGTT GTGAAAATAT TCTGCTGAAT 2520

GCTGCCTGGC TAAAGCGAGA TGCTGAGAGC ATCCACCAGT ACCTCCTTCA GCGGAAGGCC 2580

TCACCACCCA CAGTGTGAAT CCTGAGGTGC CGCCATTGGC TTCTGCTGCT TCGCTGCAGG 2640

GATAAGAACC CCTCTTCATC CCACTCTGGA CCCCAGCGGT GCTGACCCAC ATGGACTGCA 2700

GCATGAATGG CTCCCTTGCC CTCTGACTTC AGCTTCTGTT TATCCAATTA TTAATATCCA 2760

AAGAAGATGA GAAGATATAA GGAAGAGATG GTCAGAGTAC TCTGGCTTTC TCATTGCCAG 2820

GTCACATGGG TTGTCTGTGT CCCTTTAGCA AAGACCACAG CTCTTACCAA GCAGCCCCCT 2880

CTAGCTTCTG GCTCCTTCTC TTTGCTCCTT CACATCTAGG AATAGAAAGA AAGGAGAACT 2940

CGAGAGCTGG GTGCGGTGCG TCACGCTTGT AATCCCAGCA CTTTGGGAGG CCGAGACGGG 3000

TAGATCACCT GAGGTCAAGA ATTCAAGACC AGCCTGGCCA ACATGGTGAA ACCCCGTCTC 3060

TACTAAAAAT ACAAAAATTA GCTGGGCATG GTGGGCCTGT GGTGATGGTT TGCATCCTTC 3120

CTCCTTTGTC CCAATAAAGT ATGGGAGGAA TTC 3153

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3362 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:839..2647
(D) OTHER INFORMATION:/product= "CDS for PSA-68"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CGAATTCCTT CAAAAGCCAC CAAAGAGGTT CACTGAGAAA TCTCTGCACC TCCCTCCCCA 60

GGTACACAGC CTCTCCTAGA TGGGCCAGCG WGACCGTCAC CTGCATGTCC TTTCAGAGAC 120

CTGTGTATAG GGGCAGAGAA GCTTTCCTTT CTCTTTTGAC CAGAACTTTC TTGGTAACCT 180

TGCTGTTTTC ATGTACCAGT GTGTACTCAG CTGCCCCGTC TCATGGTGGG AGCGTGGTGT 240

CCTGATGCAT GGGTAGACCA CAGCTTAGGT GACTTATGCC CACCTCACAC ACTTCCAGGC 300

TGTCGCCAAT CTGTCACCAT CACAGTGAAT AACCTTGTAT ATTCACCATT TTGCAGTACA 360

GATGTGGATA TATCTGTAGG ACAGATTTCT GCAATGGAAT TGCTAGGTCA GAGGGTATGG 420

CTGTAAACAT TATCTGCAAA TAAGAAAAAG ACAACCAATC CAATAAAAAT GTAGGTAAAG 480

GGTATGAACA GTTCATAGAA AAGGAACTGC AAATCGCTTG CCAGCATATA AAAATATGCT 540

CAGCCATTCA CTCATGATAA AAGAGGTGTA AATTCAAGCT ACTCCGAGAC AGTGCCACAT 600

GCCTATTGGC AACACTAAAG TGGGAAAGAC TCGTGTGTTG AGGACGCAGA GTCACTGGGT 660

CAAAAGATGG TGTGTGTGTC CGTGTTTACA CTCCTGTTGG CAAAGCAGAG CAAGGAAAAT 720

TTGCGTTAGA GGTTGCTGCT AAAACCTTGC CTTTTTATAA GGACTACTTC AATGTTCCTT 780

ATCCTCTACC TAAAATTGAT CTCATTGCTA TTGCAGACTT TGCAGCTGGT AAAGTGCCAT 840

GGAGAACTGG GGCCTTGTTA CTTATAGGGA GACTGCATTG CTTATTGATC CAAAAAATTC 900

CTGTTCTTCA TCCCGCCAGT GGGTTGCTCT GGTTGTGGGA CATGAACTCG CCCATCAATG 960

GTTTGGAAAT CTTGTTACTA TGGAATGGTG GACTCATCTT TGGTTAAATG AAGGTTTTGC 1020

ATCCTGGATT GAATATCTGT GTGTAGACCA CTGCTTCCCA GAGTATGATA TTTGGACTCA 1080

GTTTGTTTCT GCTGATTACA CCCGTGCCCA GGAGCTTGAC GCCTTAGATA ACAGCCATCC 1140

TATTGAAGTC AGTGTGGGCC ATCCATCTGA GGTTGATGAG ATATTTGATG CTATATCATA 1200

TAGCAAAGGT GCATCTGTCA TCCGAATGCT GCATGACTAC ATTGGGGATA AGGACTTTAA 1260

GAAAGGAATG AACATGTATT TAACCAAGTT CCAACAAAAG AATGCTGCCA CAGAGGATCT 1320

CTGGGAAAGT TTAGAAAATG CTAGTGGTAA ACCTATAGCA GCTGTGATGA ATACCTGGAC 1380

CAAACAAATG GGATTTCCCC TCATTTATGT GGAAGCTGAA CAGGTAGAAG ATGACAGATT 1440

ATTGAGGTTG TCCCAAAAGA AGTTCTGTGC TGGTGGGTCA TATGTTGGTG AAGATTGTCC 1500

CCAGTGGATG GTCCCTATCA CAATCTCTAC TAGTGAAGAC CCCAACCAGG CCAAACTAAA 1560

AATTCTAATG GACAAGCCAG AGATGAATGT GGTTTTGAAA AATGTCAAAC CAGACCAATG 1620

GGTGAAGTTA AACTTAGGAA CAGTTGGGTT TTATCGGACC CAGTACAGCT CTGCCATGCT 1680

GGAAAGTTTA TTACCAGGCA TTCGTGACCT TTCTCTGCCC CCTGTGGATC GACTTGGATT 1740

ACAGAATGAC CTCTTCTCCT TGGCTCGAGC TGGAATCATT AGCACTGTAG AGGTTCTAAA 1800

AGTCATGGAG GCTTTTGTGA ATGAGCCCAA TTATACTGTA TGGAGCGACC TGAGCTGTAA 1860

CCTGGGGATT CTCTCAACTC TCTTGTCCCA CACAGACTTC TATGAGGAAA TCCAGGAGTT 1920

TGTGAAAGAT GTCTTTTTCAC CTATAGGGGA GAGACTGGGC TGGGACCCCA AACCTGGAGA 1980

AGGTCATCTC GATGCACTCC TGAGGGGCTT GGTTCTGGGA AAACTAGGAA AAGCAGGACA 2040

TAAGGCAACG TTAGAAGAAG CCCGTCGTCG GTTTAAGGAC CACGTGGAAG GAAAACAGAT 2100

TCTCTCCGCT GATCTGAGGA GTCCTGTCTA TCTGACTGTT TTGAAGCATG GTGATGGCAC 2160

TACTTTAGAT ATTATGTTAA AACTTCATAA ACAAGCAGAT ATGCAAGAAG AGAAAAACCG 2220

AATCGAAAGA GTCCTTGGCG CTACTCTTTT GCCTGACCTG ATTCAAAAAG TCCTCACGTT 2280

TGCACTTTCA GAAGAGGTAC GTCCACAGGA CACTGTATCG GTAATTGGTG GAGTAGCTGG 2340

AGGCAGCAAG CATGGTAGGA AAGCTGCTTG GAAATTCATA AAGGACAACT GGGAAGAACT 2400

TTATAACCGA TACCAGGGAG GATTCTTAAT ATCCAGACTA ATAAAGCTAT CAGTTGAGGG 2460

ATTTGCAGTT GATAAAATGG CTGGAGAGGT TAAGGCTTTC TTCGAGAGTC ACCCAGCTCC 2520

TTCAGCTGAG CGTACCATCC AGCAGTGTTG TGAAAATATT CTGCTGAATG CTGCCTGGCT 2580

AAAGCGAGAT GCTGAGAGCA TCCACCAGTA CCTCCTTCAG CGGAAGGCCT CACCACCCAC 2640

AGTGTGAATC CTGAGGTTGC GCCATTGGCG GTTCTGCTCG TTCGCTGCAG GGATAAGGTG 2700

GAGCTACCGA ACAGCTGATT CATATGCCAA GAATTTGGAG TCTTCTTTCA AACCAGTGGG 2760

GGTTGGACAA TGAATGTAGT TAACTGGTTC CTGCTCACAC TCCAGAATTA AATTCTATTG 2820

AAAAAGGAAA ATCAGCAATT CAGCAAAAAA TAAATAAAAA ATAAAAATGT AAATATGATA 2880

GTAATAAAAT AGAGCATAAC GAAACTGTGA AACTTTCTGA AGCCTTGTCA GTGGTTAAAA 2940

GTATTTAACA CTCTACTGTT AATGACAGAT GTTCTGTTTT TATAACCTAC CAAAAGGAAA 3000

CTAGAGGCTT CTTGGTGAAG AGCATTTTTG TGAAGTGGGT TCTGCAAGGA GCCTATAAAG 3060

CCAAGGGTGG TGTCCATTTC TGGGAATGGT TAAACACAAA AGGCTGATAG CTGGTATCAC 3120

ATAGTTGGAG TCAGTGCATA ATTCCAAGTG GCTTTTTTTT TTTTTGGCAC GGGGACTGAT 3180

CAGGAAGATA TATTCCTGCA TAACTCAATC TGAACCAAGG ATTGTAGTTT AGTTTTCCTC 3240

CTTGCCTTCC CTTCTGTGTG ACCGACCCCT TGGCCAAAAA AAAAACAAAA AGCAAAAAAC 3300

AAAAACCTAC CCTGTTCTGG TTTTTTTCCT CCCTTTAGTT CCACCCCCAA CCCCCGGAAT 3360

TC 3362

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3075 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION:106..2868

(D) OTHER INFORMATION:/product= "murine PSA-99"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
CCGCACCGCC GCCCGCCCGC CCGCCCGGCC CAGGCACCCG CTGGGTCTGT CCCCCGCCCC      60

AGCCTCCCCG GCGGCTCTCG GCAGGCGGTC GCCCGCGCCC GGTGA ATG TGG CTG          114
                                                  Met Trp Leu
                                                    1

GCA GCC GCC GTC CCC TCC CTC GCT CGC CGC CTG CTC CTC CTC GGC CCT        162
Ala Ala Ala Val Pro Ser Leu Ala Arg Arg Leu Leu Leu Leu Gly Pro
      5                  10                  15

CCG CCT CCT CCC CTC CTT CTC CTC CTC AGC CGC TCC TCT CGC CGC CGC        210
Pro Pro Pro Pro Leu Leu Leu Leu Leu Ser Arg Ser Ser Arg Arg Arg
 20                  25                  30                  35

CGC CGC CTC CAC AGC CTG GGC CTC GCC GCG ATG CCG GAG AAG CGG CCC        258
Arg Arg Leu His Ser Leu Gly Leu Ala Ala Met Pro Glu Lys Arg Pro
                 40                  45                  50

TTC GAG CGG CTG CCC GCC GAG GTG TCG CCC ATC AAC TAC AGC CTC TGC        306
Phe Glu Arg Leu Pro Ala Glu Val Ser Pro Ile Asn Tyr Ser Leu Cys
             55                  60                  65

CTC AAG CCC GAT TTG CTG GAC TTC ACC TTC GAG GGC AAG CTG GAG GCC        354
Leu Lys Pro Asp Leu Leu Asp Phe Thr Phe Glu Gly Lys Leu Glu Ala
         70                  75                  80

GCC GCC CAG GTA AGG CAG GCA ACC AAT CAA ATT GTG ATG AAT TGT GCT        402
Ala Ala Gln Val Arg Gln Ala Thr Asn Gln Ile Val Met Asn Cys Ala
     85                  90                  95
```

```
GAT ATT GAC ATT ATT ACA GCT TCA TAT GCC CCA GAA GGA GAT GAA GAA      450
Asp Ile Asp Ile Ile Thr Ala Ser Tyr Ala Pro Glu Gly Asp Glu Glu
100                 105                 110                 115

ATC CAT GCG ACA GGA TTT AAC TAT CAG AAT GAA GAT GAG AAA GTC ACC      498
Ile His Ala Thr Gly Phe Asn Tyr Gln Asn Glu Asp Glu Lys Val Thr
                120                 125                 130

TTG TCT TTT CCT AGC ACT CTG CAA ACA GGT ACA GGA ACC TTA AAG ATA      546
Leu Ser Phe Pro Ser Thr Leu Gln Thr Gly Thr Gly Thr Leu Lys Ile
            135                 140                 145

GAT TTT GTT GGA GAG CTG AAT GAC AAA ATG AAA GGT TTC TAT AGA AGC      594
Asp Phe Val Gly Glu Leu Asn Asp Lys Met Lys Gly Phe Tyr Arg Ser
        150                 155                 160

AGA TAC ACC ACC CCT GCC GGC GAG GTG CGC TAT GCT GCT GTC ACA CAG      642
Arg Tyr Thr Thr Pro Ala Gly Glu Val Arg Tyr Ala Ala Val Thr Gln
    165                 170                 175

TTT GAG GCT ACT GAT CCG CGA AGG GCT TTT CCT TGC TGG GAT GAG CCT      690
Phe Glu Ala Thr Asp Pro Arg Arg Ala Phe Pro Cys Trp Asp Glu Pro
180                 185                 190                 195

GCT ATC AAA GCA ACT TTT GAT ATC TCG CTG GTT GTG CCT AAA GAC AGA      738
Ala Ile Lys Ala Thr Phe Asp Ile Ser Leu Val Val Pro Lys Asp Arg
                200                 205                 210

GTG GCT TTA TCA AAT ATG AAT GTA ATT GAC AGG AAA CCA TAT CCT GAT      786
Val Ala Leu Ser Asn Met Asn Val Ile Asp Arg Lys Pro Tyr Pro Asp
            215                 220                 225

GAT GAA AAT TTA GTG GAA GTG AAG TTT GCT CGC ACA CCT GTT ATG TCT      834
Asp Glu Asn Leu Val Glu Val Lys Phe Ala Arg Thr Pro Val Met Ser
        230                 235                 240
```

```
ACG TAT CTG GTG GCA TTT GTT GTG GGT GAA TAT GAC TTT GTA GAA ACA      882
Thr Tyr Leu Val Ala Phe Val Val Gly Glu Tyr Asp Phe Val Glu Thr
    245                 250                 255

AGG TCA AAA GAT GGT GTG TGT GTC CGT GTT TAC ACC CCT GTT GGC AAA      930
Arg Ser Lys Asp Gly Val Cys Val Arg Val Tyr Thr Pro Val Gly Lys
260                 265                 270                 275

GCA GAG CAA GGA AAG TTT GCG CTC GAG GTT GCT GCT AAG ACC TTG CCT      978
Ala Glu Gln Gly Lys Phe Ala Leu Glu Val Ala Ala Lys Thr Leu Pro
                280                 285                 290

TTT TAT AAA GAC TAC TTC AAT GTT CCT TAT CCT CTA CCT AAA ATT GAT     1026
Phe Tyr Lys Asp Tyr Phe Asn Val Pro Tyr Pro Leu Pro Lys Ile Asp
            295                 300                 305

CTC ATT GCT ATT GCT GAC TTT GCA GCT GGT GCC ATG GAG AAC TGG GGC     1074
Leu Ile Ala Ile Ala Asp Phe Ala Ala Gly Ala Met Glu Asn Trp Gly
        310                 315                 320

CTT GTT ACT TAT AGG GAA ACG GCC TTG CTT ATT GAT CCA AAA AAC TCG     1122
Leu Val Thr Tyr Arg Glu Thr Ala Leu Leu Ile Asp Pro Lys Asn Ser
    325                 330                 335

TGT TCT TCA TCA CGC CAG TGG GTT GCT CTG GTT GTG GGA CAT GAA CTC     1170
Cys Ser Ser Ser Arg Gln Trp Val Ala Leu Val Val Gly His Glu Leu
340                 345                 350                 355

GCC CAT CAA TGG TTT GGA AAT CTT GTT ACT ATG GAA TGG TGG ACT CAT     1218
Ala His Gln Trp Phe Gly Asn Leu Val Thr Met Glu Trp Trp Thr His
                360                 365                 370

CTC TGG TTG AAT GAA GGC TTT GCA TCC TGG ATT GAG TAT CTT TGT GTA     1266
Leu Trp Leu Asn Glu Gly Phe Ala Ser Trp Ile Glu Tyr Leu Cys Val
            375                 380                 385
```

```
GAC CAC TGC TTT CCA GAG TAT GAT ATC TGG ACT CAG TTT GTT TCT GCA      1314
Asp His Cys Phe Pro Glu Tyr Asp Ile Trp Thr Gln Phe Val Ser Ala
        390                 395                 400

GAT TAT ACC CGT GCC CAG GAA CTT GAT GCC TTA GAT AAC AGC CAT CCT      1362
Asp Tyr Thr Arg Ala Gln Glu Leu Asp Ala Leu Asp Asn Ser His Pro
    405                 410                 415

ATT GAA GTC AGT GTG GGC CAT CCG TCT GAG GTT GAT GAG ATA TTT GAT      1410
Ile Glu Val Ser Val Gly His Pro Ser Glu Val Asp Glu Ile Phe Asp
420                 425                 430                 435

GCT ATA TCA TAT AGC AAA GGT GCA TCT GTA ATC CGA ATG CTA CAT GAC      1458
Ala Ile Ser Tyr Ser Lys Gly Ala Ser Val Ile Arg Met Leu His Asp
                440                 445                 450

TAC ATT GGT GAT AAG GAC TTT AAG AAA GGA ATG AAT ATG TAT TTA ACC      1506
Tyr Ile Gly Asp Lys Asp Phe Lys Lys Gly Met Asn Met Tyr Leu Thr
            455                 460                 465

AAG TTC CAA CAA AAG AAT GCT GCC ACA GAG GAT CTC TGG GAA AGT TTG      1554
Lys Phe Gln Gln Lys Asn Ala Ala Thr Glu Asp Leu Trp Glu Ser Leu
        470                 475                 480

GAA AGT GCC AGT GGC AAA CCC ATA GCA GCT GTG ATG AAT ACC TGG ACC      1602
Glu Ser Ala Ser Gly Lys Pro Ile Ala Ala Val Met Asn Thr Trp Thr
    485                 490                 495

AAA CAA ATG GGA TTC CCT CTC ATT TAT GTG GAA GCT GAA CAG GTA GAA      1650
Lys Gln Met Gly Phe Pro Leu Ile Tyr Val Glu Ala Glu Gln Val Glu
500                 505                 510                 515

GAT GAC AGA GTG CTG AAG CTG TCT CAG AAG AAG TTT TGT GCC AGT GGA      1698
Asp Asp Arg Val Leu Lys Leu Ser Gln Lys Lys Phe Cys Ala Ser Gly
                520                 525                 530
```

```
CCA TAT GGC GGT GAA GAC TGT CCT CAG TGG ATG GTT CCT ATC ACA ATT      1746
Pro Tyr Gly Gly Glu Asp Cys Pro Gln Trp Met Val Pro Ile Thr Ile
            535                 540                 545

TCA ACT AGT GAG GAT CCT AAC CAG GCT AAG CTG AAA ATA CTA ATG GAT      1794
Ser Thr Ser Glu Asp Pro Asn Gln Ala Lys Leu Lys Ile Leu Met Asp
        550                 555                 560

AAG CCA GAG ATG AGT GTG GTT TTG AAA AAT GTC AAA CCA GAC CAA TGG      1842
Lys Pro Glu Met Ser Val Val Leu Lys Asn Val Lys Pro Asp Gln Trp
    565                 570                 575

GTA AAG CTA AAT CTG GGA ACA GTT GGG TTT TAT CGA ACC CAG TAC AGC      1890
Val Lys Leu Asn Leu Gly Thr Val Gly Phe Tyr Arg Thr Gln Tyr Ser
580                 585                 590                 595

TCT GCC ATG CTC GAA AGT TTA TTA CCA GGC ATC CGT GAC CTT TCT CTG      1938
Ser Ala Met Leu Glu Ser Leu Leu Pro Gly Ile Arg Asp Leu Ser Leu
                600                 605                 610

CCC CCA GTG GAT CGA CTT GGA TTA CAG AAT GAC CTC TTT TCT CTG GCT      1986
Pro Pro Val Asp Arg Leu Gly Leu Gln Asn Asp Leu Phe Ser Leu Ala
            615                 620                 625

CGA GCT GGC ATC ATT AGC ACT GTA GAG GTT CTA AAA GTC ATG GAG GCT      2034
Arg Ala Gly Ile Ile Ser Thr Val Glu Val Leu Lys Val Met Glu Ala
        630                 635                 640

TTT GTG AAT GAG CCC AAT TAT ACT GTA TGG AGC GAC CTG AGC TGT AAC      2082
Phe Val Asn Glu Pro Asn Tyr Thr Val Trp Ser Asp Leu Ser Cys Asn
    645                 650                 655

CTG GGG ATT CTT TCA ACT CTC TTG TCC CAC ACA GAC TTC TAT GAG GAA      2130
Leu Gly Ile Leu Ser Thr Leu Leu Ser His Thr Asp Phe Tyr Glu Glu
660                 665                 670                 675
```

ATC CAG GAG TTT GTC AAA GAT GTC TTT TCA CCT ATA GGA GAG AGA TTG 2178
Ile Gln Glu Phe Val Lys Asp Val Phe Ser Pro Ile Gly Glu Arg Leu
680 685 690

GGC TGG GAC CCC AAA CCT GGA GAA GGT CAT CTA GAC GCA CTC CTG AGG 2226
Gly Trp Asp Pro Lys Pro Gly Glu Gly His Leu Asp Ala Leu Leu Arg
695 700 705

GGC TTG GTG CTG GGC AAA CTT GGA AAA GCA GGC CAT AAG GCA ACT TTG 2274
Gly Leu Val Leu Gly Lys Leu Gly Lys Ala Gly His Lys Ala Thr Leu
710 715 720

GAA GAA GCC CGT CGT CGG TTT AAG GAG CAC GTG GAA GGG AAA CAG ATT 2322
Glu Glu Ala Arg Arg Arg Phe Lys Glu His Val Glu Gly Lys Gln Ile
725 730 735

CTT TCT GCT GAC CTA AGG AGT CCT GTC TAT CTC ACT GTT TTA AAG CAT 2370
Leu Ser Ala Asp Leu Arg Ser Pro Val Tyr Leu Thr Val Leu Lys His
740 745 750 755

GGG GAT GGC GCT ACC TTA GAT ATC ATG CTG AAG CTT CAC AAA CAA GCT 2418
Gly Asp Gly Ala Thr Leu Asp Ile Met Leu Lys Leu His Lys Gln Ala
760 765 770

GAT ATG CAA GAA GAG AAA AAC AGA ATT GAA AGA GTT CTT GGG GCT ACT 2466
Asp Met Gln Glu Glu Lys Asn Arg Ile Glu Arg Val Leu Gly Ala Thr
775 780 785

CTT TCA CCT GAA CTG ATT CAA AAA GTC CTT ACT TTT GCA CTT TCA GAA 2514
Leu Ser Pro Glu Leu Ile Gln Lys Val Leu Thr Phe Ala Leu Ser Glu
790 795 800

GAG GTC CGT CCG CAG GAC ACT GTG TCG GTG ATT GGT GGA GTG GCT GGA 2562
Glu Val Arg Pro Gln Asp Thr Val Ser Val Ile Gly Gly Val Ala Gly
805 810 815

```
GGC AGC AAG CAT GGG AGG AAA GCT GCT TGG AAA TTC ATC AAG GAC AAC      2610
Gly Ser Lys His Gly Arg Lys Ala Ala Trp Lys Phe Ile Lys Asp Asn
820                 825                 830                 835

TGG GAG GAG CTT CAC AAC CGG TAC CAG GGA GGG TTC TTA ATA TCC AGA      2658
Trp Glu Glu Leu His Asn Arg Tyr Gln Gly Gly Phe Leu Ile Ser Arg
                840                 845                 850

CTA ATA AAG CTC TCA GTT GAG GGG TTT GCA GTT GAT AAA ATG GCT GGA      2706
Leu Ile Lys Leu Ser Val Glu Gly Phe Ala Val Asp Lys Met Ala Gly
            855                 860                 865

GAA GTT AAG GCT TTC TTC GAG AGT CAC CCA GCT CCT TCA GCT GAG CGC      2754
Glu Val Lys Ala Phe Phe Glu Ser His Pro Ala Pro Ser Ala Glu Arg
        870                 875                 880

ACC ATC CAG CAG TGT TGT GAA AAT ATC CTG CTG AAT GCT GCT TGG CTC      2802
Thr Ile Gln Gln Cys Cys Glu Asn Ile Leu Leu Asn Ala Ala Trp Leu
    885                 890                 895

AAG CGA GAT GCT GAC AGC ATT CAC CAG TAC CTC CTT CAG CGG AAA ACC      2850
Lys Arg Asp Ala Asp Ser Ile His Gln Tyr Leu Leu Gln Arg Lys Thr
900                 905                 910                 915

TCC CCA CCC TCG GTG TGA GGCCTCCTCC GCACCTGGGC CTCACTGCCT             2898
Ser Pro Pro Ser Val
                920

CTCTGCAGGG ATGAGGTGGA GCTACCAAAC AGCTGACTCA CATGCCAAGA ATCTGGAGTC    2958

TTACACCAGT GGGGGTTGGA CAATGAATGT AGTAGGTCCC TGCTCACACT CCAGAATTAA    3018

ATTCTATTGA AAAAGGAAAA TCAGCAGTTC AGCAAAAAAA AAAAAAAAAA AAAAAAA       3075
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 920amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Trp Leu Ala Ala Ala Val Pro Ser Leu Ala Arg Arg Leu Leu Leu
1 5 10 15

Leu Gly Pro Pro Pro Pro Pro Leu Leu Leu Leu Leu Ser Arg Ser Ser
20 25 30

Arg Arg Arg Arg Arg Leu His Ser Leu Gly Leu Ala Ala Met Pro Glu
35 40 45

Lys Arg Pro Phe Glu Arg Leu Pro Ala Glu Val Ser Pro Ile Asn Tyr
50 55 60

Ser Leu Cys Leu Lys Pro Asp Leu Leu Asp Phe Thr Phe Glu Gly Lys
65 70 75 80

Leu Glu Ala Ala Ala Gln Val Arg Gln Ala Thr Asn Gln Ile Val Met
85 90 95

Asn Cys Ala Asp Ile Asp Ile Ile Thr Ala Ser Tyr Ala Pro Glu Gly
100 105 110

Asp Glu Glu Ile His Ala Thr Gly Phe Asn Tyr Gln Asn Glu Asp Glu
115 120 125

Lys Val Thr Leu Ser Phe Pro Ser Thr Leu Gln Thr Gly Thr Gly Thr
130 135 140

Leu Lys Ile Asp Phe Val Gly Glu Leu Asn Asp Lys Met Lys Gly Phe
145 150 155 160

Tyr Arg Ser Arg Tyr Thr Thr Pro Ala Gly Glu Val Arg Tyr Ala Ala
165 170 175

Val Thr Gln Phe Glu Ala Thr Asp Pro Arg Arg Ala Phe Pro Cys Trp
180 185 190

Asp Glu Pro Ala Ile Lys Ala Thr Phe Asp Ile Ser Leu Val Val Pro
195 200 205

Lys Asp Arg Val Ala Leu Ser Asn Met Asn Val Ile Asp Arg Lys Pro
210 215 220

Tyr Pro Asp Asp Glu Asn Leu Val Glu Val Lys Phe Ala Arg Thr Pro
225 230 235 240

Val Met Ser Thr Tyr Leu Val Ala Phe Val Val Gly Glu Tyr Asp Phe
245 250 255

Val Glu Thr Arg Ser Lys Asp Gly Val Cys Val Arg Val Tyr Thr Pro
260 265 270

Val Gly Lys Ala Glu Gln Gly Lys Phe Ala Leu Glu Val Ala Ala Lys
275 280 285

Thr Leu Pro Phe Tyr Lys Asp Tyr Phe Asn Val Pro Tyr Pro Leu Pro
290 295 300

Lys Ile Asp Leu Ile Ala Ile Ala Asp Phe Ala Ala Gly Ala Met Glu
305 310 315 320

Asn Trp Gly Leu Val Thr Tyr Arg Glu Thr Ala Leu Leu Ile Asp Pro
325 330 335

Lys Asn Ser Cys Ser Ser Ser Arg Gln Trp Val Ala Leu Val Val Gly
340 345 350

His Glu Leu Ala His Gln Trp Phe Gly Asn Leu Val Thr Met Glu Trp
355 360 365

Trp Thr His Leu Trp Leu Asn Glu Gly Phe Ala Ser Trp Ile Glu Tyr
370 375 380

Leu Cys Val Asp His Cys Phe Pro Glu Tyr Asp Ile Trp Thr Gln Phe
385 390 395 400

Val Ser Ala Asp Tyr Thr Arg Ala Gln Glu Leu Asp Ala Leu Asp Asn
405 410 415

Ser His Pro Ile Glu Val Ser Val Gly His Pro Ser Glu Val Asp Glu
420 425 430

Ile Phe Asp Ala Ile Ser Tyr Ser Lys Gly Ala Ser Val Ile Arg Met
435 440 445

Leu His Asp Tyr Ile Gly Asp Lys Asp Phe Lys Lys Gly Met Asn Met
450 455 460

Tyr Leu Thr Lys Phe Gln Gln Lys Asn Ala Ala Thr Glu Asp Leu Trp
465 470 475 480

Glu Ser Leu Glu Ser Ala Ser Gly Lys Pro Ile Ala Ala Val Met Asn
485 490 495

Thr Trp Thr Lys Gln Met Gly Phe Pro Leu Ile Tyr Val Glu Ala Glu
500 505 510

Gln Val Glu Asp Asp Arg Val Leu Lys Leu Ser Gln Lys Lys Phe Cys
515 520 525

Ala Ser Gly Pro Tyr Gly Gly Glu Asp Cys Pro Gln Trp Met Val Pro
530 535 540

Ile Thr Ile Ser Thr Ser Glu Asp Pro Asn Gln Ala Lys Leu Lys Ile
545 550 555 560

Leu Met Asp Lys Pro Glu Met Ser Val Val Leu Lys Asn Val Lys Pro
565 570 575

Asp Gln Trp Val Lys Leu Asn Leu Gly Thr Val Gly Phe Tyr Arg Thr
580 585 590

Gln Tyr Ser Ser Ala Met Leu Glu Ser Leu Leu Pro Gly Ile Arg Asp
595 600 605

Leu Ser Leu Pro Pro Val Asp Arg Leu Gly Leu Gln Asn Asp Leu Phe
610 615 620

Ser Leu Ala Arg Ala Gly Ile Ile Ser Thr Val Glu Val Leu Lys Val
625 630 635 640

Met Glu Ala Phe Val Asn Glu Pro Asn Tyr Thr Val Trp Ser Asp Leu
645 650 655

Ser Cys Asn Leu Gly Ile Leu Ser Thr Leu Leu Ser His Thr Asp Phe
660 665 670

Tyr Glu Glu Ile Gln Glu Phe Val Lys Asp Val Phe Ser Pro Ile Gly
675 680 685

Glu Arg Leu Gly Trp Asp Pro Lys Pro Gly Glu Gly His Leu Asp Ala
690 695 700

Leu Leu Arg Gly Leu Val Leu Gly Lys Leu Gly Lys Ala Gly His Lys
705 710 715 720

Ala Thr Leu Glu Glu Ala Arg Arg Arg Phe Lys Glu His Val Glu Gly
725 730 735

Lys Gln Ile Leu Ser Ala Asp Leu Arg Ser Pro Val Tyr Leu Thr Val
740 745 750

Leu Lys His Gly Asp Gly Ala Thr Leu Asp Ile Met Leu Lys Leu His
755 760 765

Lys Gln Ala Asp Met Gln Glu Glu Lys Asn Arg Ile Glu Arg Val Leu
770 775 780

Gly Ala Thr Leu Ser Pro Glu Leu Ile Gln Lys Val Leu Thr Phe Ala
785 790 795 800

Leu Ser Glu Glu Val Arg Pro Gln Asp Thr Val Ser Val Ile Gly Gly
805 810 815

Val Ala Gly Gly Ser Lys His Gly Arg Lys Ala Ala Trp Lys Phe Ile
820 825 830

Lys Asp Asn Trp Glu Glu Leu His Asn Arg Tyr Gln Gly Gly Phe Leu
835 840 845

Ile Ser Arg Leu Ile Lys Leu Ser Val Glu Gly Phe Ala Val Asp Lys
850 855 860

Met Ala Gly Glu Val Lys Ala Phe Phe Glu Ser His Pro Ala Pro Ser
865 870 875 880

Ala Glu Arg Thr Ile Gln Gln Cys Cys Glu Asn Ile Leu Leu Asn Ala
885 890 895

Ala Trp Leu Lys Arg Asp Ala Asp Ser Ile His Gln Tyr Leu Leu Gln
900 905 910

Arg Lys Thr Ser Pro Pro Ser Val
915 920

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TTAATGCAGA AGTACATCGG 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AGGGCTTGTC TATCCCTCAC 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TCCGCCACAG AACATCTTAG 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CAGGTTCCGC CACAGAACAT 20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TTTAGTCTTC CAGAATCCAA 20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AGCGCGGGCC ACCGCCGGAG 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GCCAGCCACA TCCACCGAGC 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGCAGCTGCC AGCCACATCC 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CCTCCTCTCC GGCATCGCGG 20

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AAGGGCCTCT TCTCCGGCAT 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GCGGCCTCCA GCTTGCCCTC 20

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

ATCTCCTTCT GGTGCATATG 20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CACCTGGGCG GCGGCCTCCA 20

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TGCTCTCAGC ATCTCGCTTT 20

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CGCAACCTCA GGATTCACAC 20

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TTAACTACAT TCATTGTCCA 20

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TTTTCCTTTT TCAATAGAAT 20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GCTCTATTTT ATTACTATCA 20

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ACTGACAAGG CTTCAGAAAG 20

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CCAAGAAGCC TCTAGTTTCC 20

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TAGGCTCCTT GCAGAACCCA 20

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TTATGCACTG ACTCCAACTA 20

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

TCCTGATCAG TCCCCGTGCC 20

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TATCTTCCTG ATCAGTCCCC 20

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GCAAGGAGGA AAACTAAACT 20

Claims

1. An isolated protein having puromycin-sensitive aminopeptidase (PSA) activity comprising a polypeptide selected from the group consisting of the polypeptide with the amino acid sequence set forth in SEQ ID NO:2, and the polypeptide with the amino acid sequence set forth in SEQ ID NO:6.

2. An isolated protein according to claim 1 with the amino acid sequence set forth in SEQ ID NO:2.

3. An isolated protein which is an amino acid mutant or a fragment of a protein according to claim 1, or a derivative of said amino acid mutant, fragment or protein according to claim 1.

4. A fragment according to claim 3 selected from the group consisting of PSA-93 with the amino acid sequence extending from amino acids 51 to 875 in SEQ ID NO:2, and PSA-68 with the amino acid sequence extending from amino acids 274 to 875 in SEQ ID NO:2.

5. A process for the preparation for a protein according to claim 1 comprising chemical synthesis, recombinant DNA techniques, or a combination of these methods.

6. A process according to claim 5 comprising recombinant DNA techniques characterized in that suitably transformed host cells producing a protein according to claim 1 are multiplied in vitro or in vivo.

7. Use of a protein according to claim 1 for the generation of anti-PSA antibodies.

8. Antibody generated against a protein according to claim 1 which specifically recognizes and binds to a protein according to claim 1.

9. Antibody according to claim 8 which is a monoclonal antibody.

10. A cell line producing an antibody according to claim 9.

11. An isolated nucleic acid encoding a protein according to any of claims 1 or 3, or a fragment of said nucleic acid.

12. An isolated nucleic acid according to claim 11 which is a DNA, or a fragment of such DNA.

13. An isolated nucleic acid according to claim 11 encoding the protein with the amino acid sequence set forth in SEQ ID NO:2, or a fragment thereof.

14. An isolated nucleic acid according to claim 13 which has the nucleic acid sequence set forth in SEQ ID NO: 1, or a fragment of such nucleic acid.

15. An isolated nucleic acid according to claim 11 encoding the protein with the amino acid sequence set forth in SEQ ID NO:6, or a fragment of such nucleic acid.

16. An isolated nucleic acid according to claim 11 wherein the protein is PSA-93 with the amino acid sequence extending from amino acids 51 to 875 in SEQ ID NO:2, and PSA-68 with the amino acid sequence extending from amino acids 274 to 875 in SEQ ID NO:2.

17. An isolated nucleic acid molecule encoding PSA which isolated nucleic acid molecule is capable of hybridizing under stringent conditions to a nucleic acid molecule with the nucleotide sequence set forth in SEQ ID NO:1, or to a fragment of this nucleic acid molecule comprising about 14 or more contiguous nucleotides that are the same as (or complementary to) any about 14 or more contiguous nucleotides of the nucleic acid with the sequence set forth in SEQ ID NO: 1.

18. A method for identifying nucleic acid encoding PSA comprising contacting mammalian DNA with a probe which is a nucleic acid or fragment according to claim 11, and identifying DNA(s) which under stringent conditions specifically hybridize(s) to said probe.

19. A process for the preparation of a nucleic acid according to claim 11 comprising chemical synthesis, recombinant DNA technology or polymerase chain reaction, or a combination of these methods.

20. A hybrid vector comprising an isolated nucleic acid according to claim 11 which nucleic acid is operably linked to suitable control sequences.

21. A host cell transfected with a hybrid vector according to claim 20.

22. A host cell capable of producing a protein according to claim 1 and containing a heterologous nucleic acid encoding said protein.

23. A method for identifying a compound capable of binding to PSA, said method comprising employing a protein according to claim 1 or 3 in a binding assay.

24. A method for identifying a compound or agent which modulates the biological activity of PSA, said method comprising contacting a protein according to any of claims 1 or 3 with at least one compound or agent, whose ability to modulate the activity of PSA is sought to be investigated, and determining the change of PSA activity of said protein caused by the component or agent.

25. A method according to claim 24 comprising contacting cells producing functionally active PSA and containing heterologous DNA encoding PSA with at least one compound to be tested for its ability to modulate the activity of PSA, and monitoring said cells for a resulting change in PSA activity.

26. A method of inducing apoptosis in a cell comprising introducing into said cell a compound or signal directly or indirectly interfering with PSA activity.

27. A method of reducing the viability of a proliferating mammalian cell or cell population exhibiting PSA activity comprising down regulating the expression level or substantially inhibiting the activity of PSA in the proliferating cell or cell population.

28. A compound inducing apoptosis in a cell which compound specifically modulates the activity of PSA in said cell and which is identified by the method of claim 25.

29. A compound reducing the viability of a proliferating mammalian cell or cell population exhibiting PSA activity which compound specifically modulates the activity of PSA in said cell and which is identified by the method of claim 25.

30. A method of modulating the expression of PSA comprising contacting tissues or cells containing the PSA gene with an oligonucleotide or oligonucleotide derivative comprising from 5 to 50 nucleotide units specifically hybridizable with selected DNA or RNA deriving from the PSA gene.

31. A method of diagnosing conditions associated with PSA expression comprising contacting cells or tissues or body fluids from an animal suspected of having a condition associated with PSA expression with an oligonucleotide or an oligonucleotide derivative, or a salt thereof where salt-forming groups are present, specifically hybridizable with selected DNA or RNA deriving from the PSA gene, and determining whether hybridization occurs.

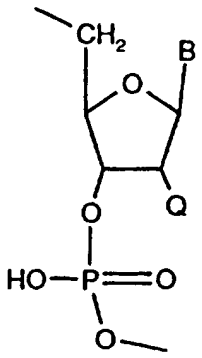
32. An oligonucleotide or an oligonucleotide derivative, or a salt thereof where salt-forming groups are present, which is specifically hybridizable with RNA deriving from the gene that encodes functional PSA, particularly PSA with the amino acid sequence set forth in SEQ ID NO:6, and preferably PSA with the amino acid sequence set forth in SEQ ID NO:2.

33. An oligonucleotide or an oligonucleotide derivative, or a salt thereof where salt-forming groups are present, according to claim 32 that is specifically hybridizable with RNA deriving from the gene that encodes PSA, comprising analogues of nucleotide units sufficient in number and identity to allow such hybridization, or a salt of said oligonucleotide derivative where salt-forming groups are present.

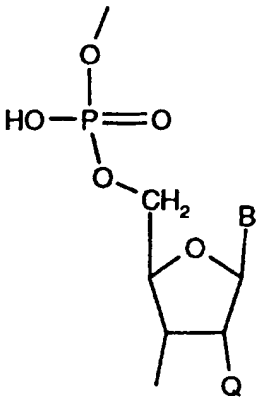
34. An oligonucleotide or oligonucleotide derivative, or a salt thereof where salt-forming groups are present, according to claim 32, which is specifically hybridizable with a particular target sequence identified in Example 3 with respect to the nucleic acid sequence set forth in SEQ ID NO:1.

35. An oligonucleotide or oligonucleotide derivative according to claim 32 comprising 5 to 50 nucleotide units.

36. An oligonucleotide or oligonucleotide derivative, or a salt thereof where salt-forming groups are present, according to claim 32 with any of the sequences given in SEQ ID NOs 7 to 31, or an allelic variant with up to 3 nucleotide analogues that differ in the sequence of a given oligonucleotide with respect to the corresponding PSA cDNA, or a salt thereof if salt-forming groups are present, or an oligonucleotide derivative thereof, or a salt thereof if salt-forming groups are present comprising at least one building block of formula I or I*,



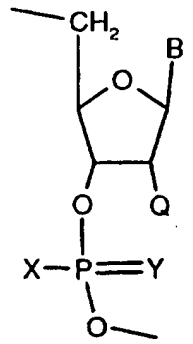
(I)



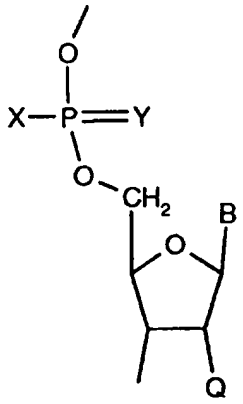
(I*)

wherein Q is H, OH, SH, SCH3, F, N_3, CN, OCN, OCH_3 or $O(CH_2)_zCH_3$, wherein z is from 1 to about 10, or $O(CH_2CHR_2O)_vR_1$, wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}alkenyl, and wherein v is from 1 to 4, and B is the base as defined in the given oligonucleotide sequence, or an analogue therof;

of formula IIa to IIf or IIa* to IIf*,



(IIa - IIf)

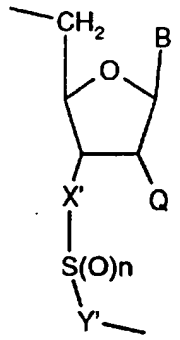


(IIa* - IIf*)

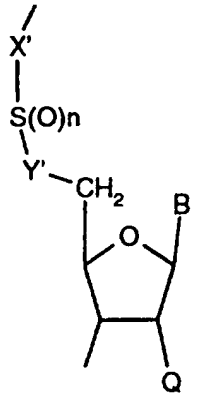
Radical of formula	type		
(IIa), (IIa*)	phosphorothioate	X = SH	Y = O
(IIb), (IIb*)	phosphorodithioate	X = SH	Y = S
(IIc), (IIc*)	methylphosphonate	X = CH_3	Y = O
(IId), (IId*)	phosphoramidate	X = NH-R	Y = O
(IIe), (IIe*)	boranophosphate	X = BH_3	Y = O
(IIf), (IIf*)	phosphotriester	X = O-R	Y = O

wherein R is lower alkyl;

of formula IIIa to IIIh or IIIa* to IIIh*



(IIIa - IIIh)

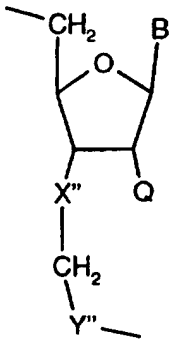


(IIIa* - IIIh*)

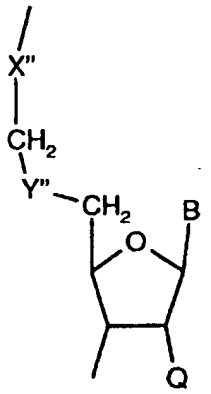
Radical of formula	type	n	X'	Y'
(IIIa), (IIIa*)	sulfate	2	O	S
(IIIb), (IIIb*	sulfonate	2	O	CH_2
(IIIc), (IIIc*)	sulfamate	2	O	NH
(IIId), (IIId*)	sulfonamide	2	NH	CH_2
(IIIe), (IIIe*)	sulfone	2	CH_2	CH_2
(IIIf),	sulfite	1	O	O

(IIIf*)				
(IIIg), (IIIg*)	sulfoxide	1	CH_2	CH_2
(IIIh), (IIIh*)	sulfide	0	CH_2	CH_2

of formula IV a to IVd or IVa* to IVd*



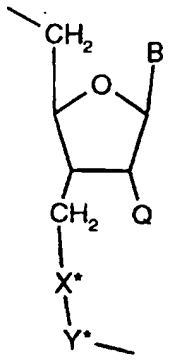
(IVa - IVd)



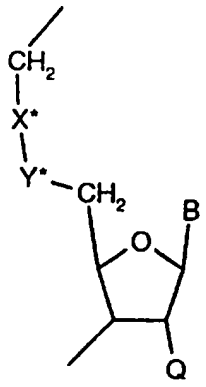
(IVa* - IVd*)

Radical of formula	type	X"	Y"
(IVa), (IVa*)	formacetal	O	O
(IVb), (IVb*)	3'-thioformacetal	S	O
(IVc), (IVc*)	5'-thioformacetal	O	S
(IVd), (IVd*)	thioether	CH_2	S

of formula Va to Vc or Va* to Vc*,



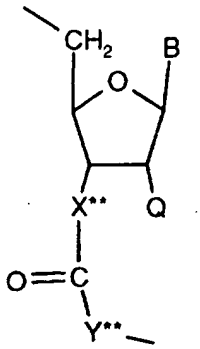
(Va - Vc)



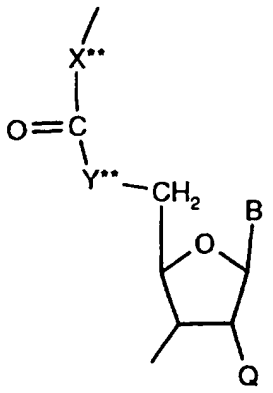
(Va* - Vc*)

Radical of formula	type	X*	Y*
(Va), (Va*)	hydroxylamine	N-H	O
(Vb), (Vb*)	methylene(methyl-imino)	N-CH_3	O
(Vc), (Vc*)	methyleneoxy(methyl-imino)	O	N-CH_3

of formula VIa to VIb or VIa* to VIb*

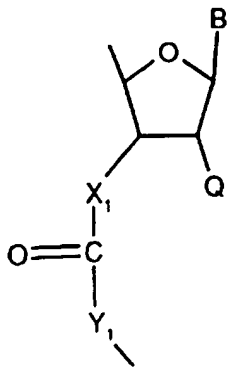


(VIa - VIb)

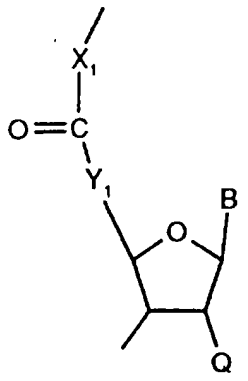


(VIa* - VIb*)

Radical of formula	type	X**	Y**
(VIa), (VIa*)	carbonate	O	O
(VIb), (VIb*)	5'-N-carbamate	O	NH
(VIc), (VIc*)	amide	CH_2	NH
(VId), (VId*)	amide II	NH	CH_2

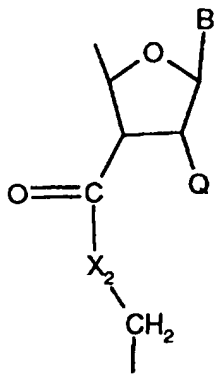


(VII)

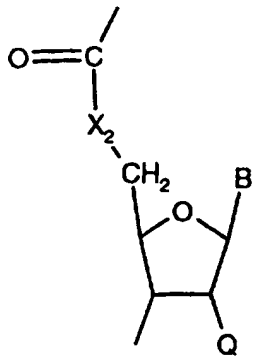


(VII*)

Radical of formula	type	X_1	Y_1
(VII), (VII*)	amide III	NH	CH_2



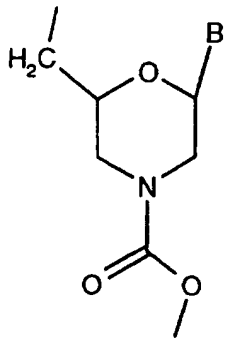
(VIII)



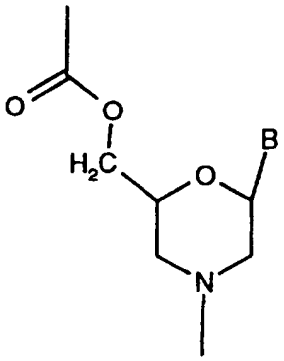
(VIII*)

Radical of formula	type	X_2

(VIII), (VIII*)	amide IV	NH

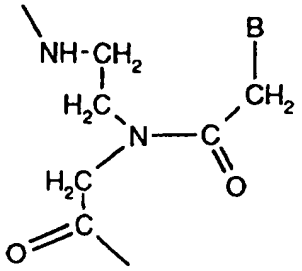


(IX)

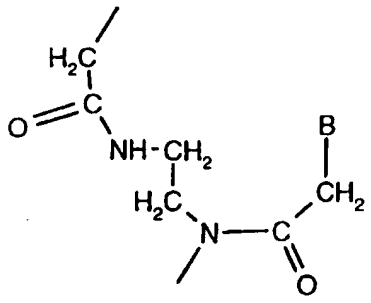


(IX*)

Radical of formula	type
IX, IX*	morpholino-carbamate



(X)



(X*)

Radical of formula	type
X, X*	peptide nucleic acid

wherein B is a base radical as defined above, Q is H, OH, SH, SCH_3, F, N_3, CN, OCN, $O(CH_2)_zNH_2$ or $O(CH_2)_zCH_3$ wherein z is from 1 to about 10, $O(CH_2CH_2O)_vCH_3$, $O(CH_2CHR_2O)_vR_1$, wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}alkenyl, wherein v is from 0 to 12; and the other moieties have the meanings given behind the respective formula.

37. An oligonucleotide derivative according to claim 36 containing only phosphorothioate building blocks of formula IIa and/or IIa*, wherein X is SH and Y is O, the central group [O-(P-SH)(=O)-O] being tautomerizable to [O-(P=S)(-OH)-O] with the more stable form depending, among others, on the solvent and the state of ionization, and wherein B have the given meanings, i.e. the meanings set forth in the sequence listing, and Q being H, or a salt thereof.

38. An oligonucleotide derivative according to claim 36 wherein at least one building block (or more) belongs to the species of formula I or I*, or IIa or IIa*, in which formulas Q is $O(CH_2CHR_2O)_vR_1$, wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, preferably hydrogen or methyl; R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}-alkenyl, R_2 preferably being hydrogen, methyl, - CH_2-OH, -or CH_2-OCH_3; wherein v is from 1 to 4, preferably from 1 to 3; B has the given meanings, i.e. the meanings set forth in the sequence listing, and all other intersugar linkages, i.e. those

which do not involve an above-defined modified sugar moiety, belong to the phosphorothioate type.

39. An oligonucleotide derivative according to claim 38 wherein the building blocks belong to the species of formula I or I*, in which formulas Q is $O(CH_2CHR_2O)_vR_1$, wherein R_1 is methyl, R_2 is hydrogen and v is 1, 2 or 3, or wherein R_1 is methyl or hydrogen, R_2 is methyl or - CH_2-OH, and v is 1.

40. An oligonucleotide derivative according to claim 36, which is a phosphorothioate type oligonucleotide derivative containing at least one amide type radical of formulas VIc, VIc*, VId, VId*, VII, VII*, VIII, or VIII*, preferably an amide- or amide III-type radical (formulas VIc, VIc* and VII, VII*, respectively), wherein B, X**, Y**, X_1 and Y_1 are as defined before, and Q is H, methoxy or methoxyethoxy ($CH_3OCH_2CH_2O$-).

41. An oligonucleotide derivative according to claim 36, which contains phosphodiester building blocks of formulas I or I* and at least one amide type radical of formulas VIc, VIc*, VId, or VId*, wherein B, X**, and Y** are as defined before, and Q is H, methoxy or methoxyethoxy (-OCH_2CH_2O CH_3).

42. A pharmaceutical composition that is suitable for administration to a warm-blooded animal suffering from a disease that responds to the modulation of PSA synthesis comprising an amount of a compound, particularly an oligonucleotide or oligonucleotide derivative, or of a salt thereof if salt-forming groups are present, according to claim 32, that is effective in the modulation of the synthesis of PSA, together with at least one pharmaceutically acceptable carrier.

43. A method of treating a disease that responds to the modulation of PSA synthesis comprising the administration of a PSA inhibitor, particularly an oligonucleotide or oligonucleotide derivative as defined in claim 32, or a pharmaceutically acceptable salt thereof, in an amount that is effective against the mentioned diseases to an animal in need of such treatment.

44. An oligonucleotide derivative, or a pharmaceutically acceptable salt thereof, according to claim 32 for the diagnostic or therapeutic treatment of a warm-blooded animal.

45. The use of an oligonucleotide derivative, or a pharmaceutically acceptable salt thereof, according to claim 32 for the preparation of a pharmaceutical composition for the treatment of tumor diseases that respond to the modulation of PSA synthesis.

46. A method of modulating the expression of PSA comprising contacting tissues or cells containing the PSA gene with an oligonucleotide or oligonucleotide derivative according to claim 32 containing the gene with an oligonucleotide derivative comprising from 5 to 50 nucleotide units specifically hybridizable with selected DNA or RNA deriving from the PSA gene.

47. A method of detecting the presence of DNA or RNA which encodes PSA in cells or tissues comprising contacting the cells or tissues with an oligonucleotide derivative according to claim 1 comprising from 5 to 50 nucleotide units specifically hybridizable with said DNA or RNA, and detecting if hybridization has occurred.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/57 C12N9/48 C07K16/40 C12N5/20 C12Q1/68
C12N5/10 C12Q1/37 A61K31/70 C07H19/167 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 45, 10 November 1995, MD US, pages 26931-26939, XP002020719 D. CONSTAM ET AL: "Puromycin-sensitive aminopeptidase"	1-35, 42-47
Y	see the whole document ---	36-41
Y	CHEMICAL REVIEWS, vol. 90, no. 4, 1 June 1990, pages 543-584, XP000141412 UHLMANN E ET AL: "ANTISENSE OLIGONUCLEOTIDES: A NEW THERAPEUTIC PRINCIPLE" see the whole document ---	36-41

-/--

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 February 1997	14.03.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Van der Schaal, C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. BUDAVARI ET AL: "The Merck Index" 1989 , MERCK&CO INC. , RAHWAY, USA XP002020720 see page 1263 no 7960 -----	28,29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 96/01518

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 7,26,27,30,31,43 and 46 are (partially) directed to a method of treatment of diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. Human PSA, antibodies against hPSA and cell lines producing them, nucleic acids encoding hPSA or hybridizing with nucleic acids encoding hPSA and their use in vectors, assays and pharmaceuticals, compounds binding to hPSA and/or modulating hPSA activity and their use (Claims 2,13 14 and 16 completely, claims 1, 3-12, 17-47 partially)

2. Murine PSA, antibodies against mPSA and cell lines producing them, nucleic acids encoding mPSA or hybridizing with nucleic acids encoding mPSA and their use in vectors, assays and pharmaceuticals, compounds binding to mPSA and/or modulating mPSA activity and their use (Claim 15 completely, claims 1, 3-12, 17-47 partially)

WO9738114

Publication Title:

PUROMYCIN-SENSITIVE AMINOPEPTIDASES

Abstract:

Abstract of WO9738114

The present invention relates to puromycin-sensitive aminopeptidases, antibodies generated against said aminopeptidases, and to means and methods for the production thereof. The invention is also directed to nucleic acids coding for said aminopeptidases, and fragments thereof, to methods of obtaining such nucleic acid molecules or fragments of the invention, and to systems suitable for the expression of such nucleic acids. One particular aspect of this invention relates to deoxyribo- and ribo-oligonucleotides and derivatives thereof, as well as pharmaceutical preparations, therapies, diagnostics and commercial research reagents in relation to disease states which respond to modulation of the synthesis of the enzyme puromycin-sensitive aminopeptidase (PSA). Data supplied from the esp@cenet database - Worldwide

---

Courtesy of http://v3.espacenet.com

This Patent PDF Generated by Patent Fetcher(TM), a service of Stroke of Color, Inc.